# SCIENTIFIC VALIDATION OF ANTI-ORAL CANCER, ANTI-TUMOUR AND ANTI-MICROBIAL ACTIVITIES OF SIDDHA METALLO-MINERAL FORMULATION " *KAALAMEGA NARAYANA CHENDHOORAM* " IN IN-VITRO STUDIES.


The dissertation Submitted by

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## GOVT. SIDDHA MEDICAL COLLEGE,

## CHENNAI-106

## DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **Scientific Validation of Anti -Oral Cancer, Anti-Tumour ,Antimicrobial Activities of *Siddha* Metallo-Mineral Formulation "*Kaalamega Narayana Chendhooram* " in Cell Line Studies** is a bonafide and genuine research work carried out by me under the guidance of **Dr.R.Karolin Daisy Rani M.D(S)., Lecturer,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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## CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **Scientific Validation of Anti-Oral cancer, Anti tumour, and Anti-microbial activities of Siddha Metallo mineral Formulation "*Kaalamega Narayana Chendhooram* " in cell line studies** is submitted to the Tamilnadu Dr.M.G.R. Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the Bonafide and genuine research work done by **Dr.R.Abinaya** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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## ENDORSEMENT BY THE HOD AND

## PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **Scientific Validation of Anti-Oral cancer, Anti tumour, and Anti-microbial activities of Siddha Metallo mineral Formulation "*Kaalamega Narayana Chendhooram* " in cell line studies** is a Bonafide work carried out by **Dr.R.Abinaya** under the guidance of **Dr.R.Karolin Daisy Rani MD(s), Lecturer,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Chennai - 106.

**Seal and Signature of the HOD** **Seal and Signature of Principal**

**Dr. M.D.Saravanadevi M.D(S)** **Dr.K.Kanakavalli M.D(S).**

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**ABBREVIATIONS**

| | |
|---|---|
| ALP | Alkaline Phosphatase |
| ALT | Alanine Transaminase |
| AST | Aspartate Amino Transferase |
| ANOVA | Analysis of Variation |
| BUN | Blood Urea Nitrogen |
| CT | Computed Tomography |
| COX | Cyclooxygenase |
| CMC | Carboxy Methyl Cellulose |
| CAMP | Cyclic Adenosine Monophosphate |
| CPCSEA | Committee for the Purpose of Control and Supervision of Experimental Animals. |
| DMEM | Dulbecco's Modified Eagle's Medium |
| DNA | DeoxyRibo Nucleic acid |
| DC | Differential Count |
| DSC | Differential Scanning Calorimeter |
| EDX | Energy Dispersive X-ray Spectrometry |
| FDG-PET | F-18 Fluoro-2-deoxy-D-glucose |
| FAD-Assay | Flavine Adenine Dinucleotide |

| | |
|---|---|
| FTIR | Fourier Transform Infrared Spectrometry |
| GOT | Glutamate Oxaloacetate Transaminase |
| GPT | Glutamate Pyruvate Transaminase |
| HPV | Human Papilloma Virus |
| HDL | High Density Lipoprotien |
| ICPMS | Inductively Coupled Plasma Mass Spectrometry |
| IAEC | Institutional Animal Ethical Committee |
| ICMR | Indian Council of Medical Research |
| LDL | Low Density Lipoprotein |
| LD50 | Lethal Dose |
| MCV | Mean Corpuscular Volume |
| MRI | Magnetic Resonance Imaging |
| MTT | 3-(4, 5-Dimethylthiazol-2-yl)-2, 5- Diphenyl Tetrazolium Bromide |
| MCH | Mean Corpuscular Haemoglobin |
| MCHC | Mean Corpuscular Haemoglobin Concentration |
| NCRP | National Cancer Registry Programme |
| OECD | Organisation for Economic Corporation and Development |
| PCR | Polymerase Chain Reaction |
| PCV | Packed Cell Volume |
| PET | Positron Emission Tomography |
| RBC | Red Blood Cells |

| | |
|---|---|
| SEM | Scanning Electron Microscope |
| SEM | Standard Error Mean |
| SGOT | Serum Glutamate Oxaloacetate |
| SGPT | Serum Glutamate Pyruvic Transaminase |
| VLDL | Very Low density Lipoprotein |
| WDS | Wavelength Dispersive Spectroscopy |
| WBC | White Blood Corpuscles |
| WHO | World Health Organization |
| XRD | X-Ray Diffraction |

# 1. INTRODUCTION

Pharmacology is the science that deals with the study of drugs, which means a drug is any substances or product that is used to modify or explore physiological systems or pathological status for the benefit of the recipient and interactions with the living systems.

The knowledge of drug usage often rested with the priest or holyman. Drugs were thought to be magical in their actions, several cultures like the Chinese, Greek, Indian, Roman, Persian, European and many others contributed to the development of medicines in early times. Though the medicines developed simultaneously in several countries. By the beginning of the 1$^{st}$ century, it was realised that there was a need to standardize the method of obtaining uniform medical preparations. With the growth of Science and development of scientific methods of research. Rigorous steps are followed and care is exercised in the introduction of new drugs. Because of the advanced scientific method of research and high incidence of cancer, I have preferred to select Anti-cancer activity. The term anti-cancer describes natural methods of health care that contributes in preventing the development of cancer or as a compliment to its conventional approaches[1].

**A bird view of oral cancer:**

***A cell is regarded as the true biological atom.***

***– George Henry Lewes***

Cells are the building blocks of life. Cell is defined as the structural and functional unit of living organisms. Our human body is made up of many tiny units called cells. The organ system is defined as group of organs that work together to carry out specific functions of the body. An organ is composed of two or more primary types of tissues. Normally, cells grow and divide to form new cells as the body needs them. When cells grow old, they die, and new cells take their place. Sometimes this orderly process goes wrong. New cells form when the body doesn't need them and old cells do not die .The extra cells can form a mass of tissue called a growth or tumour. The kinds of cells found in the tumour determine how the tumour behave. Likewise oral cavity is

also made up of cells. Sometimes changes in the oral cavity can cause precancerous conditions. These conditions may lead to a higher chance to become oral cancer[2].

***The cells of your body are like a trillion light bulbs. When you relax, purify, and connect to source, you are a powerhouse of light.***

***– Deane cooper***

Oral health is the reflection of the physiological, social factors that are essential to our quality of life. Peculiarities of the oral cavity are unique, it have a unique anatomical structure, characteristics by the juxtaposition of soft and hard tissue. Oral cavity begins at the border between the skin, lips, sides of the cheeks. The roof of the mouth is formed by the hard palate. The lowest part of the oral cavity is the floor of the mouth, which is covered by the tongue.

***Poor Oral health care can result in poor overall health.***

***– George Jaylor***

The first and foremost functions are the 1st part of digestion takes place in oral cavity, source of respiration, sound manipulation, sensory organ for taste. Due to its anatomical structure of oral cavity is continuously subject to challenge by the external environment and foreign materials. In some cases, changes in cells of oral cavity can cause cancer. Most often, Oral cavity cancer starts in flat, thin cells called squamous epithelium, which is a layer of the mucous membrane. This type of cancer is called squamous cell carcinoma of the oral cavity. [3].

***Oral health is just as important as getting a regular physical. Its not just about getting a cavity filled, its about the overall health of the individual.***

***– Jennifer Williams***

Cancer is a disease characterized by uncontrolled proliferation of cells may form a mass of tissue. Oral cancer is defined as an abnormal growth of cells in any part of the oral cavity. Oral cavity is sometimes termed as head and neck cancer.Alcohol and tobacco use including smokeless tobacco such as chewing tobacco or snuff, betel quid, human papilloma virus (HPV), especially HPV-16, is a risk factor in the oropharyngeal cancer are the most important risk factors in the cancers of head and neck. The signs and symptoms include  Lumps, nodules, White, smooth style scaly plaque appeared,

Red patches, ulcers, difficult in swallowing, inflammation and other symptoms district cannot be cured by a longer period, Oral repeated bleeding for no apparent reason, Mouth for no apparent reason numbness, burning or dryness, Difficulty in speaking or swallowing unusual.

***Bitterness is like cancer. It eats upon the host, but anger is like fire, it burns it all clean.***

***– Maya Angelo***

Among the various types of cancer oral squamous cell carcinoma is the most common type of cancer contributing to about 95% of all cancer and ranks at number six globally. OSCC remains the major cause of mortality and morbidity in patients with head and neck cancers. There are about 14.1 million of new cases estimated and 8.2 million people die out of cancer every year and 32.6 million people living with cancer (within 5 years of prevalence) in 2012 worldwide.

Now-a-days there are so many therapies and treatments are validated includes chemotherapy, radiation and surgery. Some of the most commonly used drugs for oral cancer such as Cisplatin, 5-fluorouracil (5-fu) other drugs like carboplatin, Bleomycin, Methotrexate. Prolonged use of the drugs produces certain side effects. Such as Cisplatin which is used to treat different types of cancer such as cancers of head and neck, cervical cancer, ototoxicity, neurotoxicity, electrolyte disturbances such as hypokalemia, hypomagnesiumia, hypocalcemia [5].

Whereas 5- fluorouracil produces diarrhoea, soreness, redness and peeling on the palm of the hand, sole of the feet. Bleomycin produces allergic reactions, fainting, confusion, breathing difficulties. Methotrexate produces birth defects, weakness, diarrhoea. Cetuximab produces pruritis, GIT abnormal pain, constipation, vomit.

However radiotherapy produces certain side effects such as mucositis, oral candidiasis, loss of taste, xerostomia, osteo radio necrosis of bone within the radiation field mostly in mandible, osteocytes[6].

***Healing is a matter of time, but it is sometimes also a matter of opportunity.***

***– Hippocrates***

**Siddha system of Medicine:**

As the number of people, preferring natural health remedies and herbal health remedies are increasing day by day and Indian medical systems are gaining popularity all over the world. Now-a-days, this is the best time to introduce Siddha system to the world.

***Medicine is a science of uncertainly and an art of probability.***

***- Hippocrates***

Siddha medical science is very ancient in origin, as old as the earliest civilization. The exact time of its existence cannot be as certained as it is also catagorically stated that it was before the spitting out of stand from the stone.

“கல்தோன்றி மண் தோன்றாக் காலத்தே

வாளோடு முந்தோன்றிய மூத்தகுடி”

- முதுமொழி

The word *Siddha* comes from the word Siddhi which means an object to be attained or perfection, or heavenly bliss. The Siddha medical system is the component of Dravidian system of medicine belonging to Tamil Nadu. Age of the Siddha or Tamil medical system can be dated back to 5000 B.C (History of Siddha Medicine). This system is based upon the literary evidences and oral commentaries which were passed from one generation to other. *Siddhars* who were eminent scholars and spiritual adepts documented their experiences in palm leaf manuscripts, scriptures etc., for the welfare of humanity. Of which, some were published and many remain unexplored.Generally *Siddhars* were considered to be super human beings, who have defined age and other laws of nature.

*Siddha* system of medicine is a part and parcel of the earliest Tamil medicine. It provides a cheap and efficient service to the people. The aim of this system is to keep the body and mind in a good condition. *Siddhars* had completely investigated the exact cause, effect of diseases, all kinds of drugs and thereby came to realize what was beneficial and what was not, to their existence in life.

In Siddha system oral cancer is referred as *Kanna putru* or *Vaai putru* [7]. Cancer described in the modern medical system co-relates with that of the features described in the Siddha literature under various names such as *Vippurudhi, Putru noi, Pilavai, Kazhalai* etc.

The importance and fundamental principles of siddha system of medicine were embedded in *Thirukkural,* and it clearly shows the longevity of the system not less than two thousand years. *Thiruvalluvar* in his monumental work *Thirukkural* had devoted a chapter for medicine. A deep study of those couplets threw more light on the social and medical area of those ages in Tamilnadu which gave rise to these pithy sayings,

"நோய்நாடி நோய்முதல் நாடி அதுதணிக்கும்

வாய்நாடி வாய்ப்பச் செயல்"

-திருக்குறள்

Disease is a state in which the body is not allowed to move about and the mind to think about or a state which makes the mind static and fastens the body as if every thing in a dominant state. It is pointed by *Thollkappiyar*, that the disease means suffering and depression[8].

"பையுளும் சிறுமையும் நோயின் பொருள்"

- தொல்.உரி. 34

**Role of Metals in Cancer vs Siddha System:**

***Nature is the nature of diseases.***

***– Hippocrates***

Nature has created innumerable plants, herbs, metals, poisonous substances, minerals, salts, and other organic substances. Metals are essential cellular components selected by nature to function in several indispensable biochemical process for living organisms.

***Nature itself is the best physician.***

***– Hippocrates***

Metals were endowed with unique characteristics that include redox activity, Variable co-ordination modes and reactivity towards organic substrates. Due to their reactivity metals were tightly regulated under normal conditions and aberrant metal ion concentrations were associated with various pathological disorders including cancer. The extremely diverse structural chemistry and interactions of metal complexes with bio-molecules such as nucleic acids, proteins have resulted in the exploration of anticancer activity[9]. However I would preffered to choose metal based Siddha trail drug mettalo - mineralo based ***"KAALAMEGA NARAYANA CHENDHOORAM"*** as my dissertation drug.

***Whenever the art of medicine is loved, there is also a love of humanity.***

***– Hippocrates***

**Role of Nano Medicine :**

The rich wealth of our country is selectively utilized for the welfare of mankind. The unique nature of siddha medical system is its vast and complex pharmacology.

The raw materials used in siddha medicine are of plants, metals, minerals and animal origin. The extensive use of metallo herbominsed formulations are considered as higher order.

The unique preparations of siddha system of medicines like *parpam, chendhooram, kattu*, and *padhangam* are like "life saving" and "miracle". Which were prepared by the siddhars on the basis of nano medicine.

Recent advances in science explored the nano particles, which find potential usage in bio-medical field especially in cancer.

***The aim of medicine is to prevent disease and prolong life, the ideal of medicine is to eliminate the need of a physician.***

***- William James***

Most of the medicines of the above category were found to contain nano particles and it seems siddha has used special technique to prepare each and every medicines. Coarse particles- 10000-2500nm, Fine particles-2500-100nm, Ultrafine particles 1-100nm.

***Medicine is a science of uncertainly and an art of probability.***

***- Hippocrates***

Many *siddha* nano medicines are already used in routine clinical practice and are claimed to be the very effective and potent. Nano particles exhibits unique physico-chemical properties such as ultra small size, greater surface area per weight than larger particle and reactivity. They can deliver drugs at cellular level and nuclear level. They are proved to be more effective since they improve the drug bio-availability. Nano particles medicated delivery may provide a means of alternate route, circumvenating the blood brain barrier.

With the effects of nano partical , *chendhooram* is a category of medicines made from metals or minerals (arsenicals or mercurials or salts) by grinding them with specified juices or distillates or extractives and subjecting them to a process of sublimation or calcination or burning or frying or exposing to insolation till the characteristic reddening of the product takes place. The *chendhooram* are said to retain their potency for 75 years[9].

***The good physician treats the disease, the great physician treats the patient who has the disease.***

***– Hippocrates.***

From this treasure, in-order to reduce the adverse effects of alternate medicines, less cost effectiveness, due to increased rate of prevalence among the World population, with the importance of Siddha medicine with its greater efficacy, nano partical in nature of my trial drug. I hope to strengthen the fight against oral cancer. "*KAALAMEGA NARAYANA CHENDHOORAM*" will be a valuable weapon of choice for dealing with Oral Cancer.

# 2. AIM AND OBJECTIVES

**AIM:**

To justify the ancient Siddha drug for management of oral cancer with its ultimate formulation and give good progress. The aim of this study is to establish the scientific validation of the Anti-cancer, Anti-tumor and Anti-microbial activities of **"*KAALAMEGA NARAYANA CHENDHOORAM*"** for obviously managing Oral Cancer through pre-clinical aspects. In the present medical world, there is a need for proper treatment for Oral Cancer. The aim of this study is to validate a new drug for the management of oral cancer.

**OBJECTIVES:**

The main objective of the present study is to highlight the efficacy of **"*KAALAMEGA NARAYANA CHENDHOORAM*"** on ***Oral Cancer***, the following methodology was adopted to validate the drugs and its standardization studies.

The key objectives of the study are:

- Collection of various *Siddha* and modern literature relevant to the study.
- Identification of drugs in this formulation.
- Preparation of **"*KAALAMEGA NARAYANA CHENDHOORAM*"** as per the Siddha classical text.
- Physicochemical and phytochemical investigation of the test drug.
- To evaluate bio-chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the presence of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the Acute and 28 days repeated oral Toxicity of the test drug according to OECD guidelines.
- Validation of the pharmacological study of the drug through the following activities
    - Evaluation of Anti-Cancer activity
    - Evaluation of Anti-tumor activity
    - Evaluation of Anti-microbial activity of **"*KAALAMEGA NARAYANA CHENDHOORAM*"**

# 3. REVIEW OF LITERATURE

## 3.1 DRUG REVIEW

### 3.1.1. SIDDHA ASPECT OF TRIAL DRUG

#### 1. *VEDIUPPU* (Pottasium Nitrate)

**Chemical name:** Pottasium Nitrate.

**Other names :** *Pottiluppu, Inangan, Padairasan, Boomikoormai, Navachara Mithru.*

**Preparation:**

The sand containing the crude salt is placed in a mud pot. Water is added to it and mixed well and a straw is placed inside the pot and filtered. The filtered mixture is heated to get the salt.

The Potassium nitrate salt is used for the preparation of explosives. It is also used for cooling alcohol and to polish the gold ornaments.

**General Properties:**

"மல்லாரு மட்டகுன்ம மாதருத ரக்கட்டி
கல்லா மதைப்புநீர்க் கட்டருக- லெல்லாமே
கம்பிகம்பி யென்றுங் கருவுண்டா மங்கிநின்ற
கம்பிகம்பி யென்றுரைக்குங் கால்".

-குணபாடம் தாது சீவ வகுப்பு

It cures ulcers, tumours, Urinary disorders,Ascites, *vatha* diseases.

**Purification:**

1. Salt – 100gm
2. Water – 400gm
3. Fermented butter milk – 100gm
4. Lime juice – 100 gm

Water is added to the salt and boiled on a hearth with mild flames. The white of eggs (4 nos ) is added to every 1400gm of salt and the bubbles appearing with impure substances are removed with wooden spoon.

The ingredients are then transferred to another pot, sealed with mud pasted cloth, filtered and transferred to another pot, sealed with mud pasted cloth, filtered and kept in places without aeration. Next day the water is filtered and salt is dried under sun shade. This process is repeated for seven times to get it purified.

**Properties and uses:**

- Potassium nitrate salt has got Demulcent, Diuretic And Diaphoretic properties. This should be given by dissolving in large quantities of water.
- The salt is also useful in the treatment of eight types of *Gunmam*, Uterous fibroids, Anorexia, Anaemia, Urinary Tract Infection, Dysuria, Strangulate, Ascites, Menopause disorders, Abdominal Distention and Asthma. It improves fertility in women.
- The salt is also effective in fever, swelling, rheumatic disorders, haemorrhage, gonorrhoea, eye disease and sore throat.

**Dosage:** 650 mg – 1300mg.

**This is described in the following Tamil verses:**

- The Potassium nitrate salt is also used as one of the ingredients in tooth powders.
- The Potassium nitrate salt is dissolved in water and given for the treatment of silver nitrated poisoning[10]

### 2. *PADIGARAM* (Alum )

**Chemical name:** Aluminium potassium sulphate (Alum )

**Other names :** *Cheena kaaram, cheenam, padigi*.

**Occurance:**

This is available in nature and found in combination with special form of clay in places such as Nepal, Kathiyawar, Punjab, Bihar. The alum is separated from the clay. This appears like clusters and white in colour. It has sweet, sour and also astringent in taste.

**Purification and detoxification:**

The alum is dissolved in water filtered, boiled and dried to get purified form.

**Dosage and actions:**

650mg to 1.3gm. It has astringent, antiseptic and antispasmodic in actions**.**

**General properties:**

"சீனமெனுங் காரமது சீறிவரு பல்லரணை
ஆனைக்கால் கண்ணோய் அனிலமொடு - மாநிலத்தில்
துன்மாங் கிசம்வாயு தோலாத உள்ளழலை
குன்மமிவை போக்குமெனக் கூறு".

-குணபாடம் தாது சீவ வகுப்பு

It cures gingivitis, Eye Diseases, Ophthalmia, Elephantiasis, Tumours, Sense of Heat, Gastric Ulcer, Pharyngitis, Gonorrhoea.

**Uses:**

- 35 gm of alum dissolved in 10.4ml of water is used as mouth wash and for washing the ulcers.
- In leucorrhoea with bleeding, alum is given with the juice of *Adathoda vasica* juice daily.
- 2.6gm of alum is given with sugar syrup for guinea worm infestations.
- For severe head injury 130mg of alum is administered along with sugar.
- Alum is used in hair dye preparations.

**Other preparations:**

- *Padigara parpam:*

  Absolute suppression of dysuria, stricture of urethra and *padigara chendooram*.

- *Padigara chendhuram*:

  Dysentery, blood stained dysentery, menorrhagia.

- *Padikara patru:*

  Redness of eye, lacrimated eye. The medicine is spoiled if water is added.

The alum is also one of the ingredient of *Linga thuvar* is used for control of diarrhoea and *poongavi chendhuram* for menstrual bleeding[10a].

### 3. *THURUSU* (Copper sulphate)

**Chemical name:** Copper sulphate

Copper sulphate is available in nature and also synthesized chemically. It is combined with sulphuric acid to form the copper sulphate salt which is blue in colour. When powdered it is green in colour. This is soluble in water. Lead sulphate, Pottassium nitratre, *Thottiphasanam,* Camphor, mica, alum, white *phasanam*, lead ore, toddy, rock salt, zinc, lead, soap are considered antagonistic to Copper sulphate.

Yellow arsenic trisulphide, ammonium chloride, borax, per chloride of mercury, sulphur, mercury, bismuth, cinnabar etc., are considered as agonistic to copper sulphate.

The copper available now a days in the market is not pure and it should be used only after proper purification.

**Methods of purification of *Thurusu*:**

- The impure *thurusu* (copper sulphate) is dissolved in hot water and filtered. It is then heated till the salt is formed.

- *Thurusu* is triturated with honey and ghee and boiled in a crucible. Then soaked in decanted milk / water for 3 days and dried. Thus purified *thurusu* is free from toxicity and never induces vomiting.
- The *thurusu* is placed in cow's urine and heated. It is then washed with water and dried in sunshade to get purified.
- The *thurusu* is fried with ghee, till it turns to whitish.

**General properties:**

"புண்ணாற்றுங் காமியத்தின் புண்ணாற்றுங் கண்ணோயை
விண்ணேற்று முத்தோட வீறடக்குஞ் - சண்ணுகின்ற
வாந்தியொடு பேதிதரும் வாய்நோய் சுரந்தணிக்குங்
காந்தி தருந்துரிசு காண்".

-குணபாடம் தாது சீவ வகுப்பு

**Properties and uses of *Thurusu*:**

It is a powerful astringent, emetic and antiseptic. It is an external stimulant, styptic, and mild caustic. It acts as an astringent with the dose of is 1/8 th to 2 gm weight and as an emetic with a dose of 5gm weight and it is also used in cases of poisoning.

*Thurusu* cures ulcers, eye diseases, disorders of three humors, fever and mouth diseases but causes vomiting, diarrhoea.

- *Thurusu* (325mg) mixed in 14 ml of honey is applied over the ulcers of the mouth.
- It is used for the treatments like bleeding from nose, *thurusu* 260mg is mixed in 28 ml of water and inhaled into the nose.
- *Thurusu* is powdered and dissolved in water, made it as a solution and applied for hypertrophied ulcers.

**Signs and symptoms of *thurusu* poisoning:**

Since *thurusu* dissolves in rapidly water and absorbs in blood. It produces toxic effect quickly. Over dosage of *thurusu* produces unpleasant taste, vomiting, nausea, hematemesis, blue coloured vomitus, abdominal pain, dryness of throat, excessive

thirst, jaundice, paralysis and watering of eyes. *Thurusu* poisoning may also cause death.

**Antidote for poisoning:**

- 42ml of lemon juice is given 3times daily. Alternatively ginger juice, honey and sugar may also be given together.
- Stomach wash has to be done for *thurusu* poisoning after which white of egg or milk should be given.

**Other preparations:**

- *Thurusu chenduram* - Bilious heat, bilious, nausea, tetanus, ascites, delirium.
- *Pachai ennai* -Carbuncles. ulcers[10b].

### 4. *VENGARAM (*Borax)

**Chemical name -** Sodium biborate,Borax

**Other names -** *Porikaram, Karam, Urukkinam, Urukkumithran, Danganam, Thoomathaiyadakki*

**Occurance:**

*Vengaram* is obtained abundantly in California. It is also found in Tibet and Nepal. Naturally it is obtained along with sand and dust.

**Synthetic Preparations:**

*Vengaram* is available in shops are not pure. Hence four parts of hot water and a small amount of calcium carbonate (lime) are added to it, filtered, insolated and heated till the water evaporates completely. The salt so obtained is pure and can be used. The salt appears clean white and shiny which is soluble in water and insoluble in alcohol. If it expands to air, white powder is deposited on the surface. If it is heated, the moisture evaporates and seen with minute hole.

**Actions:**

- Demulcent
- Diuretic
- Sedative
- Tonic
- Alterative
- Antiseptic
- Astringent

**General Properties:**

"சொறிபுடை யெண்குன்மநமை சோரி யாசம்
பறிகிரகணி கல்லானம் பன்னோய் - நெறியைத்
தடங்கணங்க பங்கிருமி சர்ப்பவிடஞ் சந்நி
யிடங்கணங்க லக்கிற்போ மெண்".

-குணபாடம் தாது சீவ வகுப்பு

- Toad skin
- Gastric ulcer
- Carbuncle
- Itching
- Haemorrhoids

**Purification of *Vengaram*:**

*Vengaram* is bundled and hanged in the buffalo's dung solution and boiled. The bundle is cleaned with fresh water and insolated to get it in purified form.

- It is washed in cow's dung solution
- It is soaked in buffalo's urine for 72 mins

**Uses:**

- 35gm of *Vengaram* dissolved in water of 10.4 litres is used as a mouth wash in case of oral ulcers and sore throat.
- It is also used in anal fissures and ulcers.

- Powdered *Vengaram*, sulphur, acacia catecheu ( *kaichukatti*) are taken in equal quantities, mixed with ghee and applied as a ointment for scabies, eczema, itching, and ulcer
- *Vengaram* powder is sprayed over silver fish to kill them.
- To destroy the worms which multiply in the drug, 42gm of *Vengaram* is dissolved in 11.3 litre of water and sprayed

**Other Preparations:**

*Venkkara parpam -Pitha* diseases

*Venkara maathirai* - Gastric ulcers, throbbing pain, hernia, anaemia, splenomegaly.

*Venkkara kattu* - It is used with *pudam* to get it as a *parpam*[10c].

### 5. *NAVACHAARAM (*Ammonium chloride)

**Chemical name:** Ammonium chloride

**Other names :** *Istigai, salligai, sooligai, padu.*

**General properties:**

"குன்மம் குடற்சூலை கொல்லும் மகோதரத்தை
வன்மையுறு கல்லடைப்பை மாற்றுங்காண் - சன்மக்
கவிச்சுமுத் தோடங் கனவாத நீக்கும்
நவச்சார மாதே நவில்".

-குணபாடம் தாது சீவ வகுப்பு

**General properties:**

Abdominal pain, Distended abdomen, urinary calculus, bad odour in the skin, sinusitis, amenorrhoea, whooping cough, intermittent fever, three humours, indigestion, hepatomegaly, hepatitis, splenomegaly, rhinitis, tuberculosis, haematemesis and facial palsy.

**Dosage:** 325mg to 975 mg. If given in high doses it may produce diarrhoea.

This is available in small quantities in brick stone furnace. This is also obtained by sublimation of coal, salt and dung ashes of camel. It has no smell, solid in state, fiber in nature and so it is hard to powder. It is dissoluble in water and alcohol.

**Colour:** White or grey colour.

**Taste :** Bitter, sour, urine smell.

**Synthetic preparation of *Navachaaram*:**

The sand available at the places where animals and human beings defecate is collected and placed in a pot. To one part of the sand, four parts of the urine is added; the clear liquid obtained is taken out. Camphor, alum, and potassium nitrate (3500 gm each) are powdered and burnt and added to 1300 liters of the liquid. This mixture is poured in another pot and subjected to sublimation. *Navachaaram* settles as a sublimate.

**Purification and detoxification:**

*Navachaaram* is dissolved in hot water and filtered. After it has cooled, it is poured in a broad mouthed vessel and insolated; the salt is formed in a purified form. It is preserved with small quantity of root of jequirity in a bottle.

**Other uses:**

- *Navachaaram* 4.2 g
- Alcohol 28 ml
- Rose water 560 ml
- *Navachaaram* is dissolved in alcohol and rose water mixture. A cloth is soaked in this solution and applied over the mammary gland.

**Indications:**

- Suppresion of the secretion of breast milk, breast enlargement, abscess in the breast and ulcer in the nipple.
- *Navachaaram* and potassium nitrate solution may be used for pain in the eye and excessive lacrimation

- It is used in the preparation of philosopher's liquid.

**Non-medicinal use of *Navachaaram*:**

*Navachaaram* is used for welding the metals such as tin, iron, copper and lead. It is also used in dying industry as a colouring agent.

**Other preparations:**

- *Navachaara kuzhambu* - Urinary retension, abdominal distention, anasarca, ascites.
- *Navachaara Ennai* - ascites, anasarca, eight types of gastric ulcers, jaundice.
- *Navachaara chendooram* -ulcer, colic, paralysis of the limb, dropsy, eczema
- *Navachaara kattu* - Anaemia, jaundice, white discharge[10d].

### 6.*KALLUPPU (Sodium chloride* impure)

**Chemical Name:** *Sodium chloride* impura

**Synonyms** **:** *Kadar kuruvi, Anna koormai, Arusuvai saathi, Uvaruppingunam.*

**General characters:**

"ஐயமறுஞ் சூலை யரோசிபித்தஞ் சத்தியோடு
வெய்யபிணி யட்டகுன்மம் விட்டேகும் - பெய்வளையே
வாதமதி தாகம் மலக்கட்டும் போமுலகிற்
கோதறுகல் லுப்பைக் கொடு."

-குணபாடம் தாது சீவ வகுப்பு [10e]

It is effective in the treatment of *Kapha*, Pricking pain, Loss of appetite, *Pitha* diseases, Eight types of Ulcers, *Vatha* diseases, Polydipsia and Constipation

### 7. *POONEERU( Impure Sodium Carbonate)*

**Chemical name:** *Impure Sodium Carbonate*

**Other name**: *Poovazhalai*

This is present in brackish soil. It is considered that the fuller's earth should be collected from the brackish soil during winter season and in the early morning before

the sunrise. It is collected from Sivaganga, Kalasthri, Mosur from the earth in the dew season, before sunrise.

**General properties:**

"பார்த்திட்ட பூநீற்றின் பருவங்கேளு
பங்குனியுஞ் சித்திரவை காசிக்குள்ளே
பூர்த்திட்ட ரவிசுருக்கிற் பொங்கிநீறும்
பூப்போன்மே னிற்குமதை வாரிக்கொள்ளு
-குணபாடம் தாது சீவ வகுப்பு

**Purification:**

- *Pooneeru* 1.3 litre is soaked in dew's water 5.2 litres and allow to settle. Next morning it is churned well and the outer cream layer is removed. The remaining mixture is kept in procelin plates and insolated to obtain purified form. This process is repeated for ten times and stored in a bottle.
- According to Bogar fuller's earth is dissolved in lemon juice and filtered. The filterate is boiled till the water is completely evaporatesd to get purified form.

**Uses:**

- *Pooneeru* and limestone are added in equal ratio and obtained clear water solution. The solution is used to purify the tortoise shell. egg shell pearl oyster, asbestos, forsil of crab, conch shell. The above materials are individually kept with the above said solution and boiled to get purified form. Arsenic compound may be purified with this solution.
- *Pooneeru* is mixed with the hot water. For curing, arthritis in the ankle joint and the foot is kept in the above solution for sometimes [10f].

### 8. *RASAM* ( MERCURY)

**Synonyms: Mercury or Quick Silver**

**Chemical name: Hydragyrum**

Mercury is comes under the classification of '*Pancha soothaam'*. It has many connotations such has *Sootham, Punniyam, Bharatham, Inimai, Sivasathi, Kesari* etc, according to *Dasangu nigandu.*

Mercury is obtained from its ores from countries like Spain, California, Russia, China and Japan. It is separated from its ore Cinnabar.

**Types of Mercury:**

Mercury was classified into five types.

1. *Rasam*
2. *Rasendhiran*
3. *Sootham*
4. *Misaragam*
5. *Bharatham*

**Properties:**

1. Vitalizer
2. Tonic
3. Laxative
4. Diuretic
5. Neutralising *pitham*
6. Silagogue
7. Anti-inflammatory
8. Medicine for venereal disease (*Meganasini*)

**Taste :** Six tastes dominated by sweet

**Potency:** Hot and cool (both -speciality)

**Special properties of Mercury:** Unlike other drugs Mercury is useful in the treatment of diseases caused by both heat and cold.

***Dhosam* (Impurities) of Mercury:** It is considered that there are two types of *Dhosam* of Mercury. They are

*1. Dhosam* *2. Sattai (Kavasam)*

In *Dhosam* there are 8 types of impurities in Mercury producing various diseases as shown below

| **Impurities** | **Disease caused by them** |
|---|---|
| *1. Undheenam* | *Soolai* (Throbbing pain) |
| *2. Kowdilayam* | *Kabhala noi* (Diseases of the head) |
| 3. *Anavartham* | *Biramai* (Manic illness) |
| 4. *Sangaram* | *Thathu nattam* (Spermatorrhoea) |
| 5. *Sandathvam* | *Sattium* (distress) |
| 6. *Panguthvam* | *Kuttam* (Leprosy) |
| 7. *Samalathvam* | *Moorchai* (Syncope) |
| 8. *Savisthavam* | *Sareera Elaippu* (Loss of weight) |

*Sattai* is an another type of classification, there are 7 types of impurities in Mercury which produces various diseases as shown below

| **Impurities** | **Diseases caused by them** |
|---|---|
| 1. *Naagam* | *Moolam* (Haemorrhoids) |
| 2. *Vangam* | *Tholnoikal* (Skin disease) |
| 3. *Malam* | *Arivinmai* (Idiocy) |
| 4. *Vidam* | *Maranam* (Death) |

5. *Akkini* *Thaha moham (*Polydypsia)

6. *Giri* *Sattium* (Distress)

7. *Sabalam* *Thathu nattam* (Spermatorrhoea)

**General properties of Mercury:**

"விழிநோய் கிரந்திகுன்மம் மெய்ச்சூலை புண்குட்
டழிகாலில் விந்துவினால் அத்தை – வழியாய்
புரியு விதி யாது புரியினோ யெல்லாம்
இரியுவிதி யாது மில்லை".

-குணபாடம் தாது சீவ வகுப்பு

Proper use of Mercury as a medicine has the ability to cure the following diseases they are disease in eyes, syphilis, eight types of ulcers (*gunmam*), throbbing pain (*soolai*), chronic ulcers (*perumpun*), and leprosy (*kuttam*)

**Purification and detoxification of Mercury:**

- Mercury - 35gram
- Brick powder - 100gm
- Turmeric powder - 100gm
- *Acalypha indica* juice - 1.3 lit

Mercury was triturated with finely powdered brick and with turmeric powder for one hour respectively and washed with water. Mercury is then boiled with the juice of *Acalypha indica*, it is detoxified and then finally it is washed with water thus the mercury is purified

**Other preparations of Mercury:**

- *Sootha karuppu*
- *Rasa mezhugu*
- *Rasa thailam*
- *Megavirana kalimbu*
- *Rasa kuligai*

- *Rasa parpam*
- *Mega virana kalimbu*
- *Rasa guru*
- *Rasagandhi mezhugu*
- *Rasa kattu*

**Signs and symptoms of mercurial poisoning:**

Bleeding, dropsy, anaemia, excessive body heat, sweating, diarrheoa, thirst, flatulence, blabbering, skin diseases, burning sensation of the limbs, head diseases, fever, shivering, hiccough and finally death will occur.

**Antidote for mercurial poisoning:**

- For nephrotoxicity- *Saya pattai* (dye plant) root bark is powdered and given along with jaggery.
- For loosening of teeth - the stem juice of ivy gourd (*Coccinia indica*) may be poured on the tongue.
- If there is burning sensation in limbs, urticarial, dryness of throat and unconsciousness- *Arugankizhnagu (Cynodon dactylon)* is triturated and mixed in any one of the following milk such as goat's milk, cow's milk, or cotton seed milk and administered[10g].

## 9. *LINGAM* (CINNABAR)

**Synonyms:** Natural Cinnabar, Vermilion.

**Chemical name:** Red Sulphide of Mercury.

**Other names:**

*Inkuligam, Raasam, Kadai vanni, Karpam, Kalikkam, Kaanjanam, Kaaranam, Sandagam, Samarasam, Saaniyam, Chendooram, Maniragam, Milechem, Vani and Vanni.*

Nowadays, The *Lingam* used by us is called as *Jaathi linga paadanam*, grouped under *Vaippu paadanam.*

**Preparation of *Vaippu paadanam:***

- *Rasam* (Mercury) – 280 gm
- *Gandhagam* (Sulphur) – 70 gm
- *Vediuppu* (Pottassium nitrate) – 70 gm

**Procedure:**

Mercury is thoroughly mixed and triturated with Sulphur. Potassium nitrate is then added, placed in a conical flask and burnt for 18 hours, after cooling with the red Sulphide of Mercury is collected out.

***Gunam* (Properties):**

It is hard, when it is put into fire it develops smoke; not soluble in water, has no smell and taste.

**General properties:**

"பேதிசுரஞ் சந்நி பெருவிரண நீரொடுத
காதகடி காசங் கரப்பான்புண்-ணோத
வுருவிலிங்க சங்கதமா யூறுகட்டி யும்போங்
குருவிலிங்க சங்கமத்தைக் கொள்

ஆதி யிரதவுருக் காதலாற் சாதிலிங்க
மோதி லிரதகுண முற்றுடலிற் – றீதுபுரி
குட்டங் கிரந்தி கொடுஞ்சூலை வாதமுத
லுட்டங்கு நோய்களையோட் டும்".

-குணபாடம் தாது சீவ வகுப்பு

It is effective in the treatment of diarrhoea, pyrexia, delirium,tuberculosis, scabies, unknown insect bites, syphilis, leprosy, eczema, skin diseases, throbbing pain and *vadha* diseases.

**Method of purification:**

Lime juice, cow's milk and the *Acalypha indica* juice are mixed together in equal proportion and allowed to fuse Cinnabar so as to get it in a purified potent form.

**Other preparation:**

*Sanda Rasa Parpam* - syphilis, arthritis, tremor, delirium and venereal diseases

*Padigalinga Chendooram* - dysentery, diarrhea, menorrhagia and fever

*Saathi Sambera Kuzhambu* - diarrhea, nausea, vomiting, syncope, fever and thirst

*Linga chendooram* - fever, syphilis.

*Linga kattu* - angina, syncope, anaemia, stomach ache.

**Sign and symptoms of Cinnabar toxicity:**

Dyspepsia, loss of taste, ulcers in the buccal cavity, uvula, inner portion of the tongue, larynx and large intestine, foul odour from mouth, burning sensation are the toxic symptoms of red Cinnabar.

**Antidote:**

Nutmeg (*Myristica fragrans)* - 4.2 gm

Cubeb pepper (*Piper cubeba)* - 4.2 gm

Root bark of red cotton tree *(Gossypium arboreum)* - 4.2 gm

Sugar candy - 4.2 gm,

These are mixed together and made into decoction and administered twice daily for 48 days[10h].

## 10. *THAALAGAM* ( YELLOW ORPIMENT)

**Synonyms:** Yellow Orpiment

**Chemical names:** Yellow Arsenic Trisulphide, Trisulphuret of Arsenic

**Other names:**

*Peethagi, Aalmbi, Paluppu, Kothantham, Maalam, Arithaaram, Kaalpuththi, Ponvarni, Manjal varni, Maaldevi and Arithalam*

**Types:**

Depending upon the colour, appearance and properties, *Thaalagam* has been classified into four types.

1. *Sivappu Aridharam* (Red Orpiment)
2. *Madal Aridharam*
3. *Pon Aridharam* (Gold Orpiment)
4. *Karattu Thalagam*

**General properties:**

"தாளகத்தின் பேருரைக்கத் தாலுகவுள் நோய்குஷ்டம்
நீளக் குளிர்காய்ச்சல் நீடுகபம்-நாளகங்கொள்
துஷ்டப் பறங்கிப்புண் சூழழுகண் மண்டைநோய்
கிட்டப் படுபமா கிளத்து".

-குணபாடம் தாது சீவ வகுப்பு

It is effective in the treatment of skin diseases, diseases of head and tongue, *kapha* diseases, urinary tract diseases, and venereal focus ulcer in the urethra

**Actions:**

Expectorant, antipyretic, convalescent, tonic, emetic

**Method of purification:**

*Thaalagam* is bundled and kept immersed in the mixture of lime stone, cow's urine, *Aclypha indica* juice and heated to get the purified form.

**Other medicines:**

*Thalaga ennai (Virana sanjeevi thailam)* - Heals chronic ulcers

*Thalaga karuppu* - Asthma, fever

*Thalaga maaththirai* - Fever, poisonous attack.

**Signs and symptoms of *Thaalagam* poisoning:**

*Thaalagam* if not prepared properly, it leads to be highly toxic. The following symptoms such as burning pain of the stomach, gastritis, hoarseness of voice, nasal bleeding, bleeding from the nail buds, itching over the head and redness in the tip of the hairs, mental disorders, lower abdominal swelling and throbbing pain in the lumbar region, bronchitis and sciatica are present.

**Antidote:**

| | | |
|---|---|---|
| Root Bark of Ceylon lead wort (*Plumbago zeylanica*) | _ | 8.75gm |
| Pepper (*Piper nigrum*) | – | 8.75gm |

These are added together and made into decoction. Culinary salt (4.37gm) is then added and the mixture is consumed twice daily for 21 to 42 days[10i]

### 11. *GANDHAGAM* (SULPHUR)

**Chemical name:** Sulphur, Sulphur

**Other names:**

*Kaarizhain naatham, Parai veeriam, Atheetha prakaasam, Peejam, Sakthi, Sakthi peesam, Selvi vindhu, Chendoorathaadhi, Naatham, Naatram, Deviuram, Ponvaruni.*

**General properties:**

"நெல்லிக்காய்க் கந்திக்கு நீள்பதினெண் குட்டமந்தம்
வல்லை கவிசைகுன்ம வாயுகண்ணோய் - பொல்லா
விடக்கடிவன் மேகநோய் வீறுசுரம் பேதி
திடக்கிரக ணீகபம்போந் தேர்".

-குணபாடம் தாது சீவ வகுப்பு

*Gandhagam* is bitter and astringent in taste. Its actions are laxative, tonic and antiseptic. It increases the various secretions of the body including skin. When used in high doses, it causes diarrhea.

**Types:**

*Gandhagam* is divided into four types depends upon their colour, appearance and properties.

1. White coloured Sulphur

2. Red coloured Sulphur

3. Golden yellow coloured Sulphur

4. Black coloured Sulphur.

In addition, gooseberry Sulphur and stick Sulphur (*Vaana gandhagam)* have been mentioned in most of the text books of ancient Siddha medicines. Gooseberry Sulphur is one which is often used in medicinal preparations.

**Gooseberry Sulphur (*Nellikai gandhagam):***

It is used in the treatment of 18 types of skin diseases, liver enlargement, abdominal distension, eye diseases, chronic venereal diseases, chronic diarrhea, gastric ulcer, poisonous bites, fever, and chronic dysentery.

**Method of purification:**

Sulphur is placed in an Iron spoon. A small quantity of cow's butter is added and the spoon is heated till the butter melts, this mixture is immersed in inclined position in cow's milk. This procedure is repeated for 30 times to get purified Sulphur. Fresh milk is to be used every time[10j].

## 12. *MANOSILAI* (REALGAR) RED ORPIMENT

**Synonyms:** Realgar

**Chemical names:**

Arsenic Disulphidum, Bisulphuret of Arsenic

**Types:**

*Manosilai* (Bisulphuret of Arsenic) is of two types

1. *Piravi sarakku* – It is naturally available.

2. *Vaippu sarakku* – It is obtained by adding 5 parts of Arsenic trioxide and 3 parts of Sulphur.

**General properties:**

"கொடிய குஷ்டம் காய்ச்சல் நடுக்கலஜ கல்லிரைப்
புச்சிலந்திப் பேசறும னோசிலைக்குப் பேசு".

-குணபாடம் தாது சீவ வகுப்பு

It has body strengthening and rejuvenating properties. Its potency is good. This is effective in the treatment of leprosy, fever with chills, asthma, eye diseases, urinary tract infections, *kapha* diseases and cervical adenitis.

**Method of purification:**

*Manosilai* is triturated with any one of the following juices for 3 hours, Ginger juice, lemon juice or butter milk, it is then dried to a get purified form.

**Medicinal uses:**

- It is not used alone but mostly in combination with other drugs, pills and oil.
- The oil is effective in the treatment of fistula.

**Other medicines:**

*Kasthruri karuppu, Vishnu chakkara maaththirai, Gandhaga urundai, Paashana maaththirai, Bhramanandha bhairavam, Sivanar amirtham, Sandhirodhaya maaththirai*[10j].

### 3.1.2 MODERN ASPECT OF TRIAL DRUG

### 1. POTASSIUM NITRATE

Potassium nitrate is a chemical compound with the chemical formula $KNO_3$. It is an ionic salt of potassium ions $K^+$ and nitrate ions $NO_3^-$, and is therefore an alkali metal nitrate. It occurs in nature as a mineral, niter. It is a source of nitrogen, from which it derives its name. Potassium nitrate is one of the several nitrogen-containing compounds collectively referred as salt peter or salt peter. Major uses of potassium nitrate as a fertilizers, tree stump removal, rocket propellants and fireworks. It is one of the major constituents of gun powder (black powder) and has been used since the Middle Ages as a food preservative.

**Fig no:1 Potassium nitrate**

**Etymology:**

Potassium nitrate, because of its early and global use and production, has many names. Hebrew and Egyptian words for it had the consonants n-t-r, indicating likely cognation in the Greek nitron, which was Latinised to nitrum or nitrium. Then Old French had niter and Middle English nitre. By the 15th century, Europeans referred to it as salt peter and later as nitrate of potash, as the chemistry of the compound was more fully understood.

**Properties:**

| | |
|---|---|
| Chemical formula | : $KNO_3$ |
| Molar mass | : 101.1032 g/mol |
| Appearance | : white solid |

| | |
|---|---|
| Odour | : Odourless |
| Density | : 2.109 g/cm$^3$ (16 °C) |
| Melting point | : 334 °C (633 °F; 607 K) |
| Boiling point | : decomposes at 400 °C |
| Solubility in water | : 133 g/L (0 °C), 242 g/L (20 °C), 2439 g/L (100 °C)[3] |
| Solubility | :slightly soluble in ethanol, soluble in glycerol, ammonia |
| Basicity (p$K_b$) | : 15.3[4] |
| Magnetic susceptibility ($\chi$) | : $-33.7 \cdot 10^{-6}$ cm$^3$/mol |
| Refractive index ($n_D$) | : 1.335, 1.5056, 1.5604 |
| Specific heat capacity ($C$) | : 95.06 J/mol |

**Properties:**

Potassium nitrate has an orthorhombic crystal structure at room temperature, which transforms to a trigonal system at 129 °C (264 °F).Potassium nitrate is moderately soluble in water, but its solubility increases with temperature. The aqueous solution is almost neutral, exhibiting pH6.2 at 14 °C (57 °F) for a 10% solution of commercial powder. It is not very hygroscopic, absorbing about 0.03% water in 80% relative humidity over 50 days. It is insoluble in alcohol and is not poisonous; it can react explosively with reducing agents, but it is not explosive on its own.

**Uses:**

- It is used in Thailand as main ingredient for kidney tablets to relieve the symptoms of cystitis, pyelitis and urethritis.
- Potassium nitrate is desensitise sore throat. It is used as a diuretic. It is used to cure stomach ailments to arthritis.
- It also have aphrodisiac effects.

- It reduces blood pressure and vascular diseases.
- It improves the functioning of muscles and nerves when added with sodium, creating the nervous systems electrical potential[11].

## 2. ALUMINUM SULPHATE

Aluminium sulphate, is a classical adjuvant most often used in vaccines in humans, includes a range of salts of aluminum precipitated under basic conditions, usually aluminum sulphate mixed with a sodium or potassium hydroxide plus a variable amount of phosphate. The relative proportions will determine the size, charge, and solubility of alum. The composition of alum used as an adjuvant varies in currently available vaccines and may influence vaccine immunogenicity. Alum is utilized as an adjuvant in many of the currently available vaccines composed of inactivated toxins or recombinant proteins (live attenuated vaccines do not include alum or other adjuvants). Alum, any of a group of hydrated double salts, usually consisting of aluminum sulphate, water of hydration, and the sulphate of another element. A whole series of hydrated double salts results from the hydration of the sulphate of a single charged cation (*e.g.*, $K^+$) and the sulphate of any one of a number of triple charged cations (*e.g.*, $Al^{3+}$).

Most alums have an astringent and acid taste. They are colourless, odourless, and exist as a white crystalline powder. Alums are generally soluble in hot water, and they can be readily precipitated from aqueous solutions to form large octahedral crystals.

**Fig no:2 Aluminum sulphate**

**Properties:**

| | |
|---|---|
| Taste | : Astringent |
| Colour | : colourless |
| Odour | : Odourless |

| | |
|---|---|
| Solubility | : Generally soluble in hot water |
| Molecular weight | : 258.192g/mol |
| Physical Description | : Large transparent crystals or white crystalline powder. |
| Melting point | : 92.5°C |
| Density | : 1.725 |
| PH | : 3-4 |

**Uses:**

- Alums have many uses, but they have been partly supplanted by aluminum sulphate itself, which is easily obtainable by treating bauxite ore with sulphuric acid.
- The commercial uses of alums mainly stem from the hydrolysis of the aluminum ions, which results in the precipitation of aluminum hydroxide. This chemical has various industrial uses.
- Paper is sized, for example, by depositing aluminum hydroxide in the interstices of the cellulose fibres. Aluminum hydroxide adsorbs suspended particles from water and is thus a useful flocculating agent in water-purification plants. When used as a mordant (binder) in dyeing, it fixes dye to cotton and other fabrics, rendering the dye insoluble. Alums are also used in pickling, in baking powder, in fire extinguishers, and as astringents in medicine[12].

## 3. COPPER SULPHATE

Copper sulphate, also known as cupric sulphate, or copper sulphate, is the inorganic compound with the chemical formula $CuSO_4\,(H_2O)_x$, where x can range from 0 to 5. The pentahydrate (x = 5) is the most common form. Older names for this compound include blue vitriol, bluestone, vitriol of copper and Roman vitriol.

The pentahydrate ($CuSO_4 \cdot 5H_2O$), the most commonly encountered salt, is bright blue. It exothermically dissolves in water to give the aquo complex $[Cu(H_2O)_6]^{2+}$, which has octahedral molecular geometry.

The structure of the solid pentahydrate reveals a polymeric structure where in copper is again octahedral but bound to four water ligands. The Cu (II) $(H_2O)_4$ centers

are interconnected by sulphate anions to form chains. Anhydrous copper sulphate is a white powder.

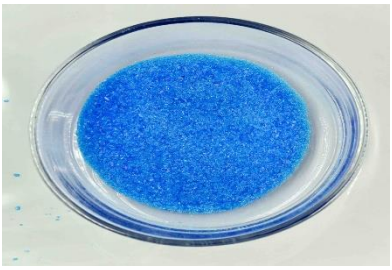


**Fig no:3 Copper sulphate**

**Properties:**

| | |
|---|---|
| Chemical formula (pentahydrate) | :$CuSO_4$ (anhydrous),$CuSO_4 \cdot 5H_2O$ |
| Molar mass (pentahydrate) | :159.609 g/mol (anhydrous), 249.685 g/mol |
| Appearance | : gray-white (anhydrous), blue (pentahydrate) |
| Solubility in water | : 1.055 molal (10 °C), 1.26 molal (20 °C), 1.502 molal (30 °C) |
| Solubility | : anhydrous insoluble in ethanol |
| Magnetic susceptibility ($\chi$) | : $+1330 \cdot 10^{-6}$ $cm^3/mol$ |
| Refractive index ($n_D$) (anhydrous) | : 1.724–1.739 (anhydrous), 1.724–1.739 |

**Uses:**

- Copper sulphate pentahydrate is used as a fungicide. However, some fungi are capable of adapting to elevated levels of copper ions. Copper sulphate is used to test blood for aneamia. The blood is tested by dropping into a solution of copper sulphate of known specific gravity –blood which contains sufficient hemoglobin sinks rapidly due to its density, whereas blood which does not sink or sinks slowly has insufficient amount of hemoglobin.

- Copper sulphate is used as an anti-fungal agent to protect seeds against fungus and to protect horse hooves from infection. It inhibits growth of bacteria such as *Escherichia coli*. Copper sulphate was used in the past as an emetic. It is now considered too toxic for this use.
- It is a antiseptic agent. Antifungal agent for topical use. Treatment for copper deficiency. Copper sulphate can be applied to your plants before disease starts as a preventative as well as when you begin to notice the dark spots of infection show.

## 4. SODIUM BORATE (BORAX)

Borax, also known as sodium borate, sodium tetraborate, or disodium tetraborate, is an important boron compound, a mineral, and a salt of boric acid. Powdered borax is white, consisting of soft colourless crystals that dissolve in water.

A number of closely related minerals or chemical compounds that differ in their crystal water content are referred to as borax, but the word is usually used to refer to the term dehydrate. Commercially sold borax is partially dehydrated.

**Fig no:4 Sodium borate**

**Etymology:**

The English word borax is Latinized: the Middle English form was boras, from Old French boras, bourras. That may have been from medieval Latin baurach (another English spelling), along with Spanish borrax and Italian borrace, in the 9th century. Another name for borax is tincal, derived from Sanskrit.

**History:**

Borax was first discovered in dry lake beds in Tibet and was imported via the Silk Road to the Arabian Peninsula in the 8th Century AD. Borax first came into

common use in the late 19th century when Francis Marion Smith's Pacific Coast Borax Company began to market and popularize a large variety of applications under the 20 Mule Team Borax trademark, named for the method by which borax was originally hauled out of the California and Nevada deserts in large enough quantities to make it cheap and commonly available.

**Properties:**

Chemical formula : $Na_2B_4O_7 \cdot 10H_2O$ or $Na_2[B_4O_5(OH)_4] \cdot 8H_2O$

Molar mass : 381.38 (decahydrate), 201.22 (anhydrate)

Appearance : white solid.

Density : 1.73 g/cm$^3$ (solid)

Melting point : 743 °C (1,369 °F; 1,016 K) anhydrate

Boiling point : 1,575 °C (2,867 °F; 1,848 K)

Magnetic susceptibility ($\chi$): $-85.0 \cdot 10^{-6}$ cm$^3$/mol

**Uses:**

- It is used to make buffer solutions in biochemistry, as a fire retardant, as an anti-fungal compound, in the manufacture of fiberglass, as a flux in metallurgy, neutron-capture shields for radioactive sources, a texturing agent in cooking, as a precursor for other boron compounds, and along with its inverse, boric acid, is useful as an insecticide. In artisanal gold mining, the borax method is sometimes used as a substitute for toxic mercury in the gold extraction process.
- Borax was reportedly and used it as gold miners in parts of the Philippines in the 1900s. Borax has been in use as an insecticide in the United States with various restrictions since 1946[14].

## 5. AMMONIUM CHLORIDE

Ammonium chloride is an inorganic compound with the formula $NH_4Cl$ and a white crystalline salt that is highly soluble in water. Solutions of ammonium chloride are mildly acidic. Sal ammoniac is a name of the natural, mineralogical form of ammonium chloride. The mineral is commonly formed on burning coal dumps from condensation of coal-derived gases. It is also found around some types of volcanic

vents. It is mainly used as fertilizer and a flavouring agent in some types of liquorice. It is the product from the reaction of hydrochloric acid and ammonia. a white or colourless, odorless, water-soluble, cubic crystalline salt with a biter taste.

**Fig no:5 Ammonium chloride**

**History:**

The earliest mention of ammonium chloride was in 554 A.D in China. At that time, ammonium chloride came from two sources: (1) the vents of underground coal fires in Central Asia, specifically, in the Tian Shan mountains (which extend from Xinjiang province of north western China through Kyrgyzstan) as well as in the Alay (or Alai) mountains of south western Kyrgyzstan, and (2) the fumaroles of the volcano Mount Taftan in south eastern Iran. (Indeed, the word for ammonium chloride in several Asian languages derives from the Iranian phrase *anosh adur* (immortal fire), a reference to the underground fires.) Ammonium chloride was then transported along the Silk Road eastwards to China and westwards to the Muslim lands and Europe. Around 800 A.D. the Arabs of Egypt discovered ammonium chloride in the soot that resulted from burning camel dung, and this source became an alternative to the sources in Central Asia.

**Properties:**

Chemical formula : $NH_3$

Appearance : Colourless

Odour : Strong pungent odour

Density : 0.86 kg / $m^3$

Melting point : 77.73°C

Solubility : Soluble in chloroform, ether, ethanol, methanol

Refractive index : 1.3327

Viscosity : 0.276

**Uses:**

- Ammonium chloride is used as an expectorant in cough medicine. Its expectorant activity is caused by irritative action on the bronchial mucosa, which causes the production of excess respiratory tract fluid, which presumably is easier to cough up.
- Ammonium salts as an irritant to the gastric mucosa and may induce nausea and vomiting.
- Ammonium chloride is used as a systemic acidifying agent in treatment of severe metabolic alkalosis, in oral acid loading test to diagnose distal renal tubular acidosis, to maintain the urine at an acid pH in the treatment of some urinary-tract disorders[15].

### 6. *SODIUM CHLORIDE IMPURE ( KALLUPPU )*

**Chemical Name** **:** *Sodium chloride impura*

**Chemical Formula** **:** NaCl (impure sodium chloride)

**Fig no:6** ***Sodium chloride impura***

**Characters:**

Black salt is type of rock salt. It is also known as Himalayas black salt. The condiment is composed largely of sodium chloride with several other components lending the salt its colour and smell. The smell is due to its sulphur content, due to the presence of iron sulphide it forms brownish pink to dark violet translucent crystals when whole and when ground into a powder it is light purple to pink in colour.

**Composition of black salt:**

It consist primary of sodium chloride and trace impurities of sodium sulphate, sodium bisulfide, sodium sulfide, iron sulphide.

Unrefined sea-salt contains small amounts of magnesium and calcium halides and sulphates and sulphates traces of algae products, salt resistance bacteria and sediment particle[16].

## 7. IMPURE OF SODIUM CARBONATE

Fuller’s earth is a naturally occurring earthy substance that has a substantial ability to adsorb impurities or colouring bodies from fats, grease, or oils. Its name originated with the textile industry, in which textile workers (or fullers) cleaned raw wool by kneading it in a mixture of water, and other contaminants from the fibres. 'Fuller's earth'" is a clay material that has the capability to decolourize oil or other liquids without chemical treatment.

Modern uses of fuller's earth include absorbents for oil, grease, and animal waste (cat litter) and as a carrier for pesticides and fertilizers.

Minor uses include filtering, clarifying, and decolourizing; active and inactive ingredient in beauty products; and as a filler in paint, plaster, adhesives, and pharmaceuticals.

**Fig no:7 Impure of sodium carbonate**

**Etymology:**

The English name reflects the historic use of the material for cleaning or "fulling" wool by textile workers called "fullers". In past centuries, fullers kneaded fuller's earth and water into woollen cloth to absorb lanolin, oils, and other greasy impurities as part of the cloth finishing process.

Fuller's Earth is also known by the following other names:

- "Bleaching clay", probably because fulling whitened the cloth.
- "Whitening clay", particularly when used to treat facial pigmentation, such as melasma.
- "Multani mitti", or "mud from Multan" in ancient India (current day Pakistan), where it was used in cosmetics.

**History:**

Fulling is an important step in the production of woolen garments, and can be traced back to ancient times. Cuneiform texts from Mesopotamia mention a raw material, im-bab-bár (Akk. gaṣṣu 'gypsum, plaster'), literally "white earth", which was delivered to fullers for the finishing of cloth. Pliny the Elder mentions several types of fuller's earth (creta fullonia in Latin) from a variety of locations, each with different properties and therefore different uses.The first references to fulling mills are from Persia, and by the time of the Crusades in the late eleventh century, fulling mills were active throughout the medieval world, the use of Fuller's Earth across the Indian subcontinent dates back to at least 1879. While its household use and transportation by

local carts in the Sindh region predates the 1800s, export by rail was first recorded in 1929 in British India.

**Composition:**

Fuller's earth consists primarily of hydrous aluminum silicates (clay minerals). Common components are montmorillonite, kaolinite and attapulgite. Small amounts of other minerals may be present in fuller's earth deposits, including calcite, dolomite, and quartz. In some localities fuller's earth refers to calcium bentonite, which is altered volcanic ash composed mostly of montmorillonite.

**Properties:**

Density: 32- 37

Specific gravity: 32- 37

PH: 6.7

**Uses:**

In addition to its original use in the fulling of raw fibers, fuller's earth is now utilized in a number of industries. Most important applications make use of the minerals natural absorbent properties in products sold as absorbents or filters.

- Treatment for poisoning. Even given the risk of salmonella, the clay content of soil could save the life of a person exposed to paraquat, for example, as paraquat is intended to break down in soil.

- Decontamination: Fuller's earth is used by military and civil emergency service personnel to decontaminate the clothing and equipment of servicemen and CBRN (chemical, biological, radiological, nuclear) responders who have been contaminated with chemical agents.
- Cleaning agent: In the Indian subcontinent, it has been used for centuries to clean marble. As a good absorbent, it removes dust, dirt, impurities and stains from the surface and replenishes the shine of the marble. It has been used numerous times to clean the Taj Mahal, India with positive results.

- Litter box: Since the late 1940s, fuller's earth has been used in commercial cat litter.
- Cosmetology and dermatology:

The same properties that make fuller's earth effective at removing oils, dirt, and impurities from wool are also effective on human hair and skin. Since ancient times it has found extensive uses in the beauty industry, both as a cosmetic and as a treatment for acne and other skin problems. Some clays have antiseptic properties, which enhance their effectiveness as skin treatments, though not all forms of fullers earth are truly antiseptic[17].

## 8. MERCURY

Mercury should not have less than 99.5 percent of Hg. It occurs naturally as a sulphide ore called Cinnabar HgS. It also occurs in small globules disseminated through rocks and as amalgam of Silver and Gold.

| | | |
|---|---|---|
| Symbol | - | Hg |
| Atomic number | - | 80 |
| Atomic mass | - | 200.59g.mol$^{-1}$ |
| Isotopes | - | 12 |

It is the only metallic element that is liquid at standard conditions of temperature and pressure[18].

**Fig no:8 MERCURY**

**Preparation:**

- It's obtained by roasting Cinnabar in a current of air $HgS+O_2 \rightarrow Hg+SO_2$
- The free Mercury gets liberated it may be either purified by volatilization or chemically by dropping Mercury into a column of dilute Nitric acid for removing basic impurities.

**Properties:**

- It has shinning silvery white in nature. Heavy liquid easily divisible into globules and extremely mobile it easy volatilizes on heating. It boils at 359.58°C.
- Almost insoluble in water, alcohol and HCl. it dissolves in cold and dilute Nitric acid, giving mercurial nitrate and Nitric oxide.

$$6Hg+8HNO_3 \rightarrow 3Hg_2 (NO_3)2+2NO+4H_2O$$

**Density:**

13.581ml at 25°C

**Mercurial preparations:**

- Mercury with Chalk (Gray powder)
- Yellow Mercuric Oxide (HgO)
- Mercuric Oxide
- Oleated Mercury
- Mercurous Chloride (HgCl-Calomel)

**Tests for Purity:**

It has been tested for weight per ml (at 25°C is about 13.5g).Non-volatile matter residue at 300°C (not more than 0.02%w/w).

**Assay:**

An accurately weighed quantity (0.49g) is dissolved in equal parts (20ml) of water and Nitric acid. It is heated gently until the solution become colourless. The

solution is then diluted with water (150ml) and sufficienct quantity of Potassium permanganate is added till a permanent pink colour is produced. A trace of Ferrous sulphate to discharge pink colour is added. Then the solution is titrated with standard 0.1N Ammonium thiocyanate (1ml of 0.1N Ammonium thiocyanate =0.01003g), using Ferric Ammonium sulphate as indicator. The temperature during the titration should not exceed above 20°C.

$3Hg+8HNO_3 \rightarrow 3Hg\ (NO_8)2+4H_20+2NO\uparrow$

$Hg\ (NO_3)2+2NH_4SCN \rightarrow 2NH_4NO_3+Hg\ (SCN)\ 2$

**Uses:**

It is a pharmaceutical aid for preparing Mercury with chalk .Formerly metallic Mercury found use as such therapeutically as a cathartic and parasiticide. But which is used as excess of has been extremely poisonous and prolonged inhalation of even very minimal amounts of Mercury prove fatal. Almost all the salts of Mercury with the exception of the Sulphide, has been poisonous.

**1. Mercury with chalk (Grew powder)**

- It is having 31 -35% w/w of Mercury and 62-70% w/w of $CaCO_3$
- It is used as a purgative (Dose 60-300mg)

**2. Yellow mercuric Oxide (HgO)**

- It is having not less than 99.5% HgO. It is used as a mild anti-septic, as anti-infective and anti- bacterial agents.

**3. Mercuric Oxide:**

- It contains not less than 95% but not more than105% w/w of the stated amount of yellow Mercuric oxide
- It is used in ophthalmology, 1% ointment to treat mild inflammatory conditions for the treatment of blepharitis and conjunctivitis.

**4. Oleated Mercury:**

- It has the equivalent of 20% of yellow Mercuric oxide
- It is used as an anti-infective.

**5. Mercuric chloride (HgCl) (Calomel):**

- It is being not less than 99.6% of HgCl
- It has been used for centuries as a cathartic but recently it is replaced by other drugs.

Calomel has been insoluble in gastric juice and has been not absorbed from the stomach. It gets absorbed in the intestine by the alkaline pancreatic juice where it slowly gets dissociated into Mercury and irritant Mercuric compounds which have been exerting a cathartic action [19].

## 9. CINNABAR

Cinnabar (red Mercury (II) Sulfide (HgS), vermilion) is the ordinary ore of Hg. It is normally found in a substantial, granular form and is bright scarlet to brick-red in colour. It is a chemical compound composed of the chemical elements Mercury and sulphur (Mercury 86.22 % Sulphur 13.78 %).

**Fig no:9 CINNABAR**

**Properties:**

| | | |
|---|---|---|
| Formula | - | Mercury (II) sulfide |
| Symbol | - | HgS |
| Molecular formula | - | HgS |
| Number | - | 32 |

| | | |
|---|---|---|
| Colour | - | brownish red and lead-grey |
| Specific gravity | - | 8.176 |
| Solubility | - | Soluble in water, |
| Molecular Weight | - | 232.66 gm |
| Melting point | - | 580 °C decomp. |
| Other anions | - | Mercuryoxide, Mercury selenide, Mercury telluride |
| Other cations | - | Zinc sulphide, Cadmium sulphide |
| Fermion Index | - | 0.26 |
| Boson Index | - | 0.74 |
| Radioactivity | - | 0GRapi i.e not radioactive (Gamma Ray American Petroleum Institute Units) |

HgS which has long been used in combination with traditional Siddha and Chinese medicine as a Sedative, Hypotonic, Ant inflammatory, Anti pyretic and Analgesic for more than 2000 years and is still widely used in Asian countries[20].

An overdose of cinnabar in drugs such as Bapuslsan, which is used as a sedative and in the management of external intoxication in the Chinese population[21].

It must be aware of its toxic effects due to high Mercury content. Previous studies have shown that the insoluble form of HgS (or) cinnabar (10 g / 1water at about 20) can still be absorbed from GIT and liver[22].

**Uses:**

- Cinnabar is the only important ore of mercury.
- The bright red pigment ‘Vermilion’ and ‘Chinese red’ are made from cinnabar[23].

**Toxic symptoms of Cinnabar:**

- Most of the soluble salts of Mercury are absorbed slowly from the intestinal mucous membrane of the alimentary tract and produce their toxic effects.
- The long term use of cinnabar containing traditional medicines could result in renal dysfunction due to accumulation of Mercury in kidney.
- Skin allergic reaction may occur when cinnabar is used in tattoo dyes.
- Blurred vision due to accumulation of Mercury in brain is possible.

## 10. YELLOW ORPIMENT

Arsenic compounds have been known since at least the days of Ancient Greece and Rome (thousands of years ago). They were used by physicians. The compound most often used for both purposes was arsenic sulphide ($As_2S_3$)[24].

Orpiment is a deep orange-yellow coloured Arsenic Sulfide mineral. Its formula is $As_2S_3$. It is formed by sublimation of Arsenic (60.90%) and Sulphur (39.10%). It takes its name from the Latin Auripigmentum (Aurum − Gold + pigmentum − pigment) because of its deep-yellow colour.

**Fig no:10 YELLOW ORPIMENT**

**Synonyms:**

*Arrhenicum Operment, Orpiment, Yellow Arsenic, Yellow Ratsbane, Auripigment, Arsenicum flavum*

**Physical Properties:**

Colour - Lemon yellow, Orange yellow

| | | |
|---|---|---|
| Density | - | 3.49 - 3.56, Average = 3.52 |
| Molecular Weight | - | 246.04 gm |
| Melting point | - | 300 °C to 325 °C |
| Diaphaneity | - | Transparent to translucent |
| Fracture | - | Sectile |
| Crystal Habit | - | Foliated |
| Hardness | - | 1.5-2 - Talc-Gypsum |
| Luminescence | - | Non-fluorescent |
| Luster | - | Pearly |
| Streak | - | Pale yellow |
| Electron Density | - | 3.23 gm / cc |
| Specific Gravity | - | 3.49 gm / cc |
| Fermion Index | - | 0.0029323747 |
| Boson Index | - | 0.9970676253 |
| Photoelectric | - | 44.66 barns / electron |
| Radioactivity | - | 0 GRapi (Gamma Ray American Petroleum Institute Units) |

**Chemical Properties:**

| | | |
|---|---|---|
| Formula | - | $As_2S_3$ |
| Elements | - | As, S |
| Common Impurities | - | Hg, Ge, Sb |

**Uses:**

- Orpiment was traded in the Roman Empire and was used as a medicine in China.
- It has been used as a fly poison and to tip arrows with poison.
- Because of its Golden colour, it was used by alchemists, both in China and the West, searching for a way to make Gold.
- It is used in the tanning industry to remove hair from hides[25].

## 11. SULPHUR:

Sulphur or Sulphur is a Greek word which means "to burn". Sulphur is a chemical element with the symbol S. It is a plentiful, multivalent non-metal. It occurs in nature as the pure element and as Sulfide and Sulphate minerals. Sulphur is referred in the Bible as brimstone (burn stone) in English. It is the sixth most abundant macro mineral in the breast milk.

**Fig no:11 SULPHUR**

**History:**

It is discovered by Chinese Before 2000BC and is recognized as an element by Antoine Lavoisier in 1777.

**General properties:**

Symbol - S

Atomic Number - 16

Element category - polyatomic nonmetal

**Physical properties:**

| | | |
|---|---|---|
| Phase | - | solid |
| Density | - | 1.96 g·cm−3 |
| Liquid density at M.P | - | 1.819 g·cm−3 |
| Heat of fusion | - | 1.727 kJ·mol−1 |
| Heat of vaporization | - | 45 kJ·mol−1 |
| Molar heat capacity | - | 22.75 J·mol−1·K−1 |
| Electronegativity | - | 2.58 (Pauling scale) |

**Chemical properties:**

| | | |
|---|---|---|
| Solubility | - | insoluble in water |
| Vanderwaals radius | - | 0.127 nm |
| Ionic radius | - | 0.184 (-2) nm; 0.029 (+6) |
| Isotopes | - | 5 |
| Electronic shell | - | [Ne] 3s23p4 |
| Standard potential | - | 0.51 V |

**Biological role:**

Sulphur is a vital component of all living cells, it is the seventh most occurring element in the human body by weight, and is used in biochemical processes. The average person takes in around 900 mg of Sulphur per day, mainly in the form of protein. In metabolic reactions, Sulphur compounds serve as both fuels and respiratory materials. Its organic form is present in the vitamins biotin and thiamine. It is an essential part of many enzymes and in antioxidant molecules.

**Uses of sulphur:**

- Organo sulphur compounds are used in pharmaceuticals and agrochemicals.
- Magnesium sulphate known as Epsom salts when in hydrated crystal form can be used as a laxative.
- Elemental sulphur is one of the oldest fungicides and pesticides.
- Sulphur is the most important fungicide in organic production.
- Octa sulphur is used in pharmaceutical skin preparations for the treatment of acne. It acts as a keratolytic agent and also kills bacteria, fungi, scabies mites and other parasites[26].

**Toxic effects of Sulphur:**

Elemental Sulphur is nontoxic, but many simple Sulphur derivatives are, such as Sulphur dioxide ($SO_2$) and Hydrogen Sulfide are toxic which include neurological effects, disturbance of blood circulation, heart damage, reproductive failure, stomach and gastrointestinal disorder, dermatological effects[27].

## 12.REALGAR ( RED ORPIMENT)

Realger, α-$As_4S_4$ is an Arsenic Sulphide mineral, also known as “Ruby Sulphur”. It is orange-red in colour, melt at $320^0$C, and burns with the bluish flame releasing fumes of Arsenic and Sulphur. It is a photosensitive mineral and will alter to Para Realger upon prolonged exposure to light. It has an Arabic name *Rahj al ghar* which means "powder of the mine."

**Other names**: Ruby Sulphur, Ruby of Arsenic.

**Fig no:12. REALGAR**

**Physical Properties:**

Formula - $As_4S_4$ or AsS

Colour - Red to yellow-orange

Density - 3.56

Diaphaneity - Transparent

Specific gravity - 3.56

Melting point - 320 °C

Molecular Weight - 106.99 gm

Refractive index - 2.538

Luminescence - Non-fluorescent

Lustre - Sub Metallic

Streak - Orange

**Electrical Properties:**

Electron Density - 3.30 gm / cc

Fermion Index - 0.0022478773

Boson Index - 0.9977521227

Radioactivity - 0 GRapi (Gamma Ray American Petroleum Institute Units)[28].

**Uses:**

The Chinese name for Realgar is *Xionghuang*, literally 'masculine yellow'. It was used to repel snakes, rats, weeds and insects, as well as being used in Chinese medication. The ancient Greeks called it as "*Sandaracha*". It is used in combination

with Potassium Chlorate to make a contact explosive known as "red explosive" for some types of torpedoes[29].

## 3.2. LITERATURE REVIEW OF DISEASE

### 3.2.1. SIDDHA ASPECT OF THE DISEASE

Siddha system of medicine deals with cancer and its treatment widely. In ancient Siddha literature, cancer is explained in the name of *Putru* which gives the direct meaning and as *Arpudham* and *Vanmeegam.* This name comes from the appearance of the cut surface of a solid malignant tumour, with "the veins stretched on all sides as the animal the crab has its feet, whence it derives its name" [30]. In Siddha system of medicine, Cancer is referred to *Vippuruthi* or *Putru.*

For the Purpose of diagnose and treatment following reference books evaluates great ideas about cancer.

1. *Yugi Vaidhya Chintamani*

2.*AnubogaVaidhyaNavaneetham*

3. *Pulipani 500* [31]

4.*Agathiya Vaidhya Vallathi*[32]

In this medical system of life, the cancerous growth and tumours are headed as *Arputhaviranangal* and *Arputhakatttikal.*

According to *Yugimamuni varvaidhya sinthamani 800* I part, some kinds of cancer clarified under different systemic diseases. *Yugi* classification of disease is compared with Western system of medicine by means of symptoms for quick and easy approach.

For example, *Ukkarasoolai* is understand as prostatic cancer

*Vilperuvayiru* is known as Testicular cancer

*Mamisamagotharam* and *Kalperuvayiru as* cancerous growth within abdomen.

Hippocrates (ca. 460 BC – ca. 370 BC) described several kinds of cancer, referring to them with the Greek word Karkinos (Crab or Crayfish). This name comes from the appearance of the cut surface of a solid malignant tumour, with "the veins stretched on all sides as the animal the crab has its feet, whence it derives its name". In Siddha system of medicine, Cancer is referred to *Vippuruth*i or *Putru.*

"புற்று நோயை மௌனப்பகைவன்
மறைந்திருந்து கொல்லும் பகைவன்".

-திருமந்திரம்

Generally, a chronic tumour or swelling or ulcer is first the identification of *Putru* in Siddha system. Tumour grows gradually and finally look like a *Putru* or cauliflower.

**Causes:**

- Taking excessive amount of salt and pungent.
- Taking large quantity of fish and meat.
- Making sleep in day time.
- Doing frequent sex.

Symptoms are varying depending on the particular type of cancer. To handle cancer effectively it is considered as *Vippuruthi*.

**Types of *Vippuruthi*:**

*Vippuruthi* is classified into seven types,

1. *Karppa Vippuruthi*

2. *Kuvalai Vippuruthi*

3. *Vatha Vippuruthi*

4. *Pitha Vippuruthi*

5. *Seththuma Vippuruthi*

6. *Santhu Vippuruthi*

7. *Oodu Vippuruthi*

**Appearance**

Causes of various classes look like one or more following appearance.

- *Kazhalaikatti*
- Spreading ulcer
- Initially like warts then grows and develops as turtle shell with oozing
- Hyper pigmentation of skin, affects hair follicles and destroys entire body.

**1. *Karppa Vippuruthi:***

Gastric regurgitation, pain in side and lower abdomen, dryness of skin, lower abdominal swelling like pregnancy and head ache.

**2. *Kuvalai Vippuruthi:***

Pain in lower back, anal region and side of the chest, fever with shivering, cough with expectoration, abdominal pain and swelling.

**3. *Vatha Vippuruthi:***

Pain and swelling in the lower abdomen, this swelling look like a frog, fever, wound in the abdomen, pus discharge from the wound and abdominal distension.

***4. Pitha Vippuruthi:***

Hematemesis, paleness of the skin, burning sensation all over the body, shivering, mental disorder , hiccup, tastelessness of the tongue, fever, dehydration, hematoma and abdominal pain.

***5. Sethuma Vippuruthi:***

Small tumour and abscess in the abdomen, abdominal pain, fever, cough and swelling of the body.

***6. Santhu Vippuruthi:***

Swelling in the side of the abdomen, this swelling is characterized by shining, hardness, cool and itching.

***7. Oodu Vippuruthi:***

Some time fever, blackish discolouration of skin, abdominal pain, giddiness, vomiting, diarrhea and body pain.

**Curable type of *Vippuruthi*:**

1. *Karpa Vippuruthi*
2. *Kuvalai Vippuruthi*
3. *Pitha Vippuruthi*
4. *Oodu Vippuruthi*

**Incurable type of *Vippuruthi*:**

1. *Santhu Vippuruthi*
2. *Sethuma Vippuruthi*
3. *Vatha Vippuruthi*[33].

### 3.2.2. MODERN ASPECT OF THE DISEASE

**Cancer:**

Cancer known medically as malignant neoplasia, is a broad group of diseases involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumours, which may invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumours are cancerous; benign tumours do not invade neighbouring tissues and do not spread throughout the body. There are over 200 different known cancers that affect humans.

**History:**

Cancer has existed for all of human history. The earliest written record regarding cancer is from circa 1600 BC in the Egyptian Edwin Smith Papyrus and describes cancer of the breast. Hippocrates (ca. 460 BC – ca. 370 BC) described several kinds of cancer, referring to them with the Greek word *Karkinos* (Crab or Crayfish). This name comes from the appearance of the cut surface of a solid malignant tumour, with "the veins stretched on all sides as the animal the crab has its feet, whence it derives its name."Galen stated that "cancer of the breast is so called because of the fancied resemblance to a crab given by the lateral prolongations of the tumour and the adjacent distended veins".

Celsus (ca. 25 BC – 50 AD) translated *Karkinos* into the Latin cancer, also meaning crab and recommended surgery as treatment. Galen (2nd century AD) disagreed with the use of surgery and recommended purgatives instead. These recommendations largely stood for 1000 years.

In the 15th, 16th and 17th centuries, it became acceptable for doctors to dissect bodies to discover the cause of death. The German professor Wilhelm Fabry believed that breast cancer was caused by a milk clot in a mammary duct.

The Dutch professor Francois de la Boe Sylvius, a follower of Descartes, believed that all disease was the outcome of chemical processes, and that acidic lymph fluid was the cause of cancer. His contemporary Nicolaes Tulp believed that cancer was a poison that slowly spreads, and concluded that it was contagious.

The physician John Hill described tobacco snuff as the cause of nose cancer in 1761. This was followed by the report in 1775 by British surgeon Percivall Pott that cancer of the scrotum was a common disease among chimney sweeps.

With the widespread use of the microscope in the 18th century, it was discovered that the 'cancer poison' spread from the primary tumour through the lymph nodes to other sites ("metastasis"). This view of the disease was first formulated by the English surgeon Campbell De Morgan between 1871 and 1874.

**Epidemiology of cancer:**

Nearly seven lakh Indians die of cancer every year, while over 10 lakh are newly diagnosed with some form of the disease. According to the latest World Cancer Report from the World Health Organisation (WHO), more women in India are being newly diagnosed with cancer annually. As against 4.77 lakh men, 5.37 lakh women were diagnosed with cancer in India in 2012.

In terms of cancer deaths, the mortality rate among men and women in India is almost the same. While 3.56 lakh men died of cancer in 2012 in India, the corresponding number for women was 3.26 lakh.

One in every 10 Indians runs the risk of getting cancer before 75 years of age, while seven in every 100 runs the risk of dying from cancer before their 75th birthday.

Cancer of lip and oral cavity has emerged as the deadliest among Indian men while for women, is breast cancer. The top five cancers in men are lip/oral cavity, lung, stomach, colourectum and pharynx, while among women they are breast, cervix, colourectum, ovary and lip/oral cavity.

The global cancer burden jumped to 14.1 million new cases in 2012, with WHO saying the marked increase in breast cancers must be addressed.

The International Agency for Research on Cancer (IARC) 2012 estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012, compared with 12.7 million and 7.6 million, respectively, in 2008.

The most commonly diagnosed cancers worldwide were those of the lung (1.8 million, 13% of the total), breast (1.7 million, 11.9%), and colourectum (1.4 million, 9.7%). The most common causes of cancer death were cancers of the lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%).

Projections based on IARC 2012 estimates predict a substantive increase to 19.3 million new cancer cases per year by 2025, due to growth and ageing of the global population. More than half of all cancers (56.8%) and cancer deaths (64.9%) in 2012

occurred in less developed regions of the world, and these percentage will increase further by 2025[34].

**Causes:**

Cancers are primarily an environmental disease with 90–95% of cases attributed to environmental factors and 5–10% due to genetics. Environmental, as used by cancer researchers, means any cause that is not inherited genetically, such as lifestyle, economic and behavioural factors, and not merely pollution.

Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and environmental pollutants.

It is nearly impossible to prove what causes a cancer in any individual, because most cancers have multiple possible causes. For example, if a person who uses tobacco heavily develops lung cancer, then it was probably caused by the tobacco use, but since everyone has a small chance of developing lung cancer as a result of air pollution or radiation, then there is a small chance that the cancer developed because of air pollution or radiation[35].

**SYMPTOMS:**

- Persistant cough
- Change in bowel habits
- Blood in the stool
- Unexplained anaemia
- Breast lump or breast discharge
- Lumps in the testicles
- Change in urination
- Haematuria (blood in urine)
- Hoarseness
- Indigestion
- Unusual vaginal bleeding

- Unexpected weight loss
- Continued itching in anal or genital area
- Non healing sores
- Back pain, Pelvic pain[36].

**Classification of cancer:**

There are five broad groups that are used to classify cancer.

1. Carcinomas are characterized by cells that cover the internal and external parts of the body such as lung, breast, and colon cancer.

2. Sarcomas are characterized by cells that are located in bone, cartilage, fat, connective tissue, muscle, and other supportive tissues.

3. Lymphomas are cancers that begin in the lymph nodes and immune system tissues.

4. Leukemias are cancers that begin in the bone marrow and often accumulate in the bloodstream.

5. Adenomas are cancers that arise in the thyroid, the pituitary gland, the adrenal gland, and other glandular tissues.

**Viruses in Human Cancer:**

Certain human malignancies are associated with viruses. Examples include Burkitt's lymphoma (Epstein-Barrvirus), Hepato cellular carcinoma (hepatitis virus), cervical cancer [Human Papilloma Virus (HPV)], and T cell leukaemia (retroviruses). The mechanisms of action of these viruses are varied but always involve activation of growth-promoting pathways or inhibition of tumour suppressor products in the infected cells.

For example, HPV proteins E6 and E7 bind and inactivate cellular tumour suppressors' p53 and pRB, respectively. Viruses are not sufficient for cancer development but constitute one alteration in the multistep process of cancer[38].

**Table no:1 TUMOUR MARKERS[38a]**

| TUMOUR MARKERS | CANCER | NON-NEOPLASTIC CONDITIONS |
|---|---|---|
| Hormones<br>• Human chorionic Gonadotropin<br>• Calcitonin<br>• Catechol amines | Gestational trophoblastic disease, gonadal germ cell tumour.<br>Medullary cancer of the thyroid.<br>Pheochromocytoma. | Pregnancy |
| Oncofetal antigens<br>• Alpha fetoprotein<br>• Carcino embryonic antigen | Hepato cellular carcinoma, gonodal germ cell tumour.<br>Adenocarcinoma of the colon, pancreas, lung, breast, ovary. | Cirrhosis, hepatitis<br>Pancreatitis, hepatitis, inflammatory bowel disease, smoking |
| Enzymes<br>Prostatic acid phosphates<br>Neuron specific enolase,<br>Lactate dehydrogenase | Prostatic cancer<br>Small cell cancer of the lung, neuroblastoma.<br>Lymphoma, Edwing's sarcoma. | Prostatitis, prostatic hypertrophy Hepatitis, hemolytic anemia |

**Metastasis:**

Metastasis is the spread of cancer to other locations in the body. The new tumours are called metastatic tumours, while the original is called the primary tumour. Almost all cancers can metastasize. Most cancer deaths are due to cancer that has spread from its primary site to other organs[39].

**Head and neck cancer:**

Tumours of the head and neck are the sixth most common malignancy in the world, with a yearly incidence of more than 500,000 cases, and it comprises approximately 4% to 5% of all new cancers and 2% of all cancer deaths (100,000 per year). Most patients are older than 50 years, and incidence increases with age; the male-to-female ratio is 2.5:1. Approximately 34% of oral and pharyngeal cancers present as localized disease, 46% present as locoregional (i.e., locally advanced or involving regional lymph nodes) disease, and 10% present as metastatic disease. Ninety percent of these cancers involve squamous cell histology. The most common sites are the oral cavity, pharynx, larynx, and hypopharynx. Nasal cavity and paranasal sinus cancers, salivary gland malignancies, and various sarcomas, lymphomas, and melanoma are less common.

**Site-specific head and neck tumours:**

**Oral cavity:**

The oral cavity includes the lip, anterior two thirds of the tongue, floor of the mouth, buccal mucosa, gingiva, hard palate, and retro molar trigone. Squamous cell carcinoma is the histologic type observed in most cases.

**Oropharynx:**

The oropharynx includes the base of the tongue, tonsils, posterior pharyngeal wall, and the soft palate.

**Larynx:**

Risk factors are history of tobacco and/or alcohol intake. In addition, certain dietary factors and exposure to wood dust, nitrogen mustard, asbestos, and nickel have been implicated as etiologic factors. The male-to-female ratio for laryngeal cancer is 4.5:1, with a peak incidence in the sixth decade of life. More than 95% of laryngeal cancers are squamous cell carcinomas.

Laryngeal cancers can be supraglottic, glottic, and/or subglottic. Early cancers not requiring laryngectomy are usually treated with radiation. If lymph nodes are involved,

neck dissection and/or neck radiation is indicated. Locally advanced resectable tumors may be treated with surgery and adjuvant radiation if locoregional risk factors are present. An alternative is the use of combined radiation and chemotherapy.

**Hypopharynx:**

Early cancers not requiring laryngectomy can be treated with surgery or radiation. Locally advanced respectable tumours may be treated with surgery followed by radiation or sequential or concomitant chemoradiation. In these cases, surgery involves total laryngectomy and partial or total pharyngectomy and neck dissection.

**Nasal Cavity and Paranasal Sinuses:**

Most tumours are squamous cell carcinomas and are usually slow growing with low incidence of metastasis. Carcinomas of the nasal cavity and paranasal sinuses are usually detected in patients in advanced stages because of the relatively silent tumour location. Treatment follows the same general guidelines as those for oral cancer.

**Nasopharynx:**

It is extremely rare in most parts of the world, with an incidence of less than 1 case per 100,000 population. However, it is endemic in certain areas, including North Africa, Southeast Asia, China, and the far northern hemisphere. EBV is strongly associated with nasopharyngeal carcinoma. This association has been demonstrated by serologic studies and by the detection of the viral genome in tumour samples. Diet (salt-cured fish and meat) and genetic susceptibility are other probable risk factors; tobacco and alcohol are not risk factors, except in a minority of cases.

**Salivary Gland Cancer:**

Salivary gland cancers make up about 3% of all head and neck cancers diagnosed in the United States yearly. Tobacco and alcohol consumption are not risk factors, except possibly in women. Ionizing radiation and certain occupational exposures (e.g., in workers of rubber and automotive industries, wood workers, and farm workers) have been associated with the development of salivary gland cancer.

The salivary glands are classified as major (parotid, submandibular, and sublingual) and minor (distributed along upper aero digestive tract, predominantly in the oral and nasal cavities and the paranasal sinuses). Most of the salivary gland cancers arise from the parotid glands; sublingual and minor salivary gland cancers are rare.

Most salivary gland tumours are benign, and the most common histology is pleomorphic adenoma, which is characterized by slow growth and few symptoms, and is most frequently seen in the parotid gland. The most common presentation of benign salivary gland tumours is asymptomatic swelling of the lip, the parotid, or the submandibular or the sublingual glands. Persistent pain or neurologic involvement (mucosal or tongue numbness and facial nerve weakness) suggests malignant disease.

Surgery is the main stay of treatment for all localized stages of salivary gland tumours. Postoperative radiation is indicated for localized tumours of high-grade histology that are large, with close or positive margins, and/or positive regional lymph nodes. Radiation is the primary treatment for unresectable tumours. The role of chemotherapy is limited to management of locally recurrent, unresectable disease or distant metastatic disease. There is no established standard chemotherapy for salivary gland cancer. Regimens employing cisplatin, carboplatin, anthracyclines, taxanes, cyclophosphamide, and 5-FU result in transient responses in 14% to 30%[40].

**Other head and neck tumours:**

**Sarcoma:**

Soft tissue sarcomas of the head and neck are relatively rare. Of head and neck sarcomas, 80% are seen in adults and 20% are in children. These tumours are heterogeneous and can present in any head and neck site, commonly as a submucosal or subcutaneous painless mass. In the hypopharynx and nasopharynx, the presenting symptoms may be cranial nerve abnormalities or airway or swallowing difficulties. As in sarcomas at other sites, grade is an important prognostic indicator. High-grade, aggressive tumours such as malignant fibrous histocytoma, angio sarcoma, osteogenic sarcoma, neuro fibrosarcoma, and soft part sarcomas tend to be locally aggressive and spread along neurovascular structures, fascia, and bone. In addition to aggressive local behaviour, there is a high risk for metastatic disease, particularly in lung, bone, central

nervous system, and liver. Metastatic disease may occur without local lymph node involvement. Sarcomas may arise after radiation therapy, but this is very uncommon in the head and neck region. The prognosis for these secondary sarcomas may be worse than for primary sarcomas.

Treatment depends on stage, age of the patient, tumour type, location and size. Wide margin resection is the goal, but may not be possible because of the proximity of vital structures. Adjuvant postoperative radiation and/or brachytherapy can improve local control in aggressive sarcomas. The major indications for adjuvant radiation are high-grade sarcomas or positive margins, lesions greater than 5 cm, and recurrent sarcoma. Elective neck radiation is not necessary because the incidence of occult positive lymph nodes is low. Soft tissue and possibly osteogenic sarcomas may benefit from adjuvant or neoadjuvant chemo radiation. Such patients should be referred to clinical trials when possible. Overall survival rate approaches 60% for patients with sarcomas of the head and neck.

**Melanoma:**

Mucosal melanomas represent less than 1.5% of all melanomas. About 50% of mucosal melanomas occur in the head and neck, and more than 20% of melanomas that occur in the head and neck region are mucosal. The age of diagnosis is 60 to 80 years. The hard palate is the most common site. Nearly one-third of these tumours are amelanotic. The proportion of mucosal melanomas is higher in African American and Hispanic populations than in white populations. Although rare in the United States, mucosal melanomas are more frequent in Japan and in some parts of Africa. Mucosal melanomas may be multiple, may have satellite lesions, may invade angio lymphatics, and can metastasize. They behave more aggressively than skin melanomas. Lymph node metastasis is observed at presentation in up to 48% of patients. Surgery is the mainstay of treatment for local or locoregional disease. Prophylactic lymph node dissection is not recommended. Radiation, when used, is usually employed adjuvantly for positive margins or used palliatively for local recurrence or unrespectability. Adjuvant use of radiation has not been shown to improve survival. Prophylactic nodal radiation is not recommended. Chemotherapy and Immunotherapy have been studied, but the effect of these interventions on survival when used as palliation or as adjuvant

therapy has not been defined. Patients should be encouraged to enter clinical trials where available. Mean overall 5-year survival is 17%[41].

**Oral cancer:**

Oral cancer is one of the more common in head and neck malignancies. Oral cancer is a general term for oral cavity cancers. It occurs in the majority were squamous cell carcinoma, which is called the mucosa mutate. In clinical practice, oral cancer, including cancer gums, tongue, hard and soft palate cancer, carcinoma of the mandible, floor of mouth cancer, oropharyngeal cancer, salivary gland cancer, lip cancer, and maxillary sinus cancer occurs in the facial skin and mucous membranes of cancer and so on.

**Causes of Oral cancer:**

**Long-term habit of tobacco, alcohol:**

Most oral cancer occurs in patients with long-term history of smoking and drinking. **Poor oral hygiene:**

Poor oral hygiene, bacteria or fungi in the mouth breeding, breeding to create the conditions, thus contributing to the formation of nitrosamines and their precursors. Coupled stomatitis, proliferation of some cells in the state, more sensitive to carcinogens, so a variety of reasons may contribute to oral cancer.

**Long-term stimulation of foreign body**:

Root or sharp cusp, inappropriate dentures long-term stimulation of oral mucosa, resulting in chronic ulcers and even cancer.

**Malnutrition:**

Vitamin A deficiency can cause oral mucosal epithelial thickening, hyperkeratosis with the occurrence of oral cancer. Demographic studies show that countries with low intake of vitamin A have high incidence of oral cancer. There are also inadequate intake of trace elements considered relevant, such as low zinc content of foods. Zinc is indispensable for the growth of animal tissue elements, zinc deficiency

may lead to mucosal epithelial damage, and create favorable conditions for the occurrence of oral cancer. Also total protein and animal protein intake may be associated with inadequate oral cancer[42].

**Leukoplakia and Erythema:**

Oral Leukoplakia and hypertrophic Erythema often a precancerous lesion.

**Associated lesions:**

**The relationship between oral cancer and precancerous lesions:**

White ulcers or blisters are often occurs in the buccal mucosa occurs, often occurs in pressure, poor sleep or eating habits (such as insufficient fruit) on the occasion, the general will heal within two weeks; If more than two weeks is not cured, must be examined to rule out the possibility of epithelial cell carcinoma.

**Changes in the oral mucosa colour:**

Normal epithelium pink, red or a white colour of polarization are not normal. If its red with white, it is more serious situation, another example of the tongue appears dark red with white dots occur like, highly suspicious of cancer.

**Ulcer:**

Over more than two weeks of oral mucosa has not yet healed.

**Clinical manifestations:**

1. Lumps, nodules;

2. White, smooth style scaly plaque appeared;

3. Red patches, ulcers, inflammation and other symptoms district cannot be cured by a longer period.

4.Repeated Oral bleeding for no apparent reason.

5. Mouth for no apparent reason numbness, burning or dryness.

6. Difficulty in speaking or swallowing unusual[43].

**Differential diagnosis:**

**Traumatic ulcers:**

This ulcers often occur in the tongue side edge, the corresponding total at the fangs and ulcers, or irregular teeth and root dental prosthesis, indicating that ulcers are caused by the stimulus. Ulcers soft, soft substrates, no induration. Eliminate these irritants 1 to 2 weeks after the ulcer to heal.

**Tubercular ulcers:**

These are almost secondary, mostly open tuberculosis direct result of the spread, often occurs in the soft palate, buccal mucosa and tongue back, shallow ulcers compared with cancerous ulcer, ulcer base induration soft non-invasive, effective anti-TB treatment. Imaging and biopsy can accurately help identify and diagnose.

**Diagnosis:**

When neck mass is the first presentated, the primary site can be located and biopsied in approximately 80% of cases. If no primary site is obvious, tissue diagnosis can be obtained by fine needle aspiration (FNA) biopsy of the node, with sensitivity and specificity approaching 99%. A non - diagnostic FNA does not rule out the presence of tumour.

Computerized tomography scan (CT scan) remains the primary imaging study for evaluation of metastatic adenopathy. Magnetic resonance imaging (MRI) may complement with CT scan. Positron emission tomography (PET) scans are being used more frequently to detect tumours that are not obvious on other scans.

Laryngoscopy and naso pharyngoscopy should be performed. With occult primary tumours, directed biopsy of the nasopharynx, tonsil, base of tongue, and pyriform sinus should be performed Bilateral tonsillectomy will sometimes reveal the source of an occult cancer[44].

**Treatment:**

The management of patients with head and neck cancer is complex. The choice of treatment modality depends on the stage and site of disease. Patients with locally advanced disease should be evaluated (prosthodontics, nutrition, speech, and swallowing) by a multidisciplinary team including otolaryngologist or head and neck surgical oncologist, radiation oncologist, medical oncologist, dentist, and personnel involved in rehabilitation before treatment is initiated[46].

In general, either surgery or radiation is effective as single-modality therapy for patients with early-stage disease (stage I or II) for most sites. The choice of modality depends on local expertise, patient preference, and functional result. For the 60% of patients with locally advanced disease (stage III, IV, and M0), combined-modality therapy is indicated.

**TREATMENT:**

- Surgery
- Radiation therapy
- Chemotherapy
- Immunotherapy
- Targeted therapy
- Hormone therapy
- Stem cell transplant, Precision medicine.

**Surgery:**

The nature of the surgical procedure is determined primarily by the size of the tumour and the structures involved. Extensive surgeries and those cancers involving function of the tongue. Frequently require myo cutaneous flaps or microvascular free flaps to achieve a more functional reconstruction.

Resectability depends on the experience of the surgeon and the rehabilitation team. In general, a tumour is unresectable if the surgeon believes that all of the gross tumour cannot be removed or that local and distant control will not be achieved after

surgery even with adjuvant radiation therapy. Generally, involvement of the skull base, pterygoid, and deep neck musculature, and of the major vessels portends a poor outcome with surgery as a primary modality.

Cervical lymph node dissections may be elective or therapeutic. Elective neck dissections are done at the time of surgery in patients with necks that are clinically negative when the risk of a positive lymph node is at least 30%. Therapeutic neck dissections are done for clinically obvious masses. This surgery is now rarely performed because of excessive morbidity, especially loss of shoulder function. The modified radical dissection preserves one or more of the nonlymphatic structures. In selective neck dissections, only certain levels of lymph nodes are removed on the basis of the specific lymphatic drainage from the primary site.

**Radiation Therapy:**

The use of radiation as a single therapy in early-stage tumours (i.e., T1 and T2) is as efficacious as surgery. The choice of therapy depends on expected quality of life, functional outcome, sequelae of therapy, and options for treatment in case of recurrence.

In locally advanced tumours (i.e., T3 and T4), radiation therapy is combined with surgery. In general, postoperative radiation is preferred over preoperative radiation according to the results of two randomized prospective studies that show superior local control and minimally increased survival in the postoperative radiation arm in hypo pharyngeal cancer patients. Postoperative radiotherapy is recommended for patients at high risk for local recurrence [i.e., T4 tumour, close or positive margins (<5 mm), perineural or perilymphatic or vascular invasion by the tumour, multiple or large positive nodes, and/or extracapsular invasion].

The radiation type varies for specific sites and for definitive versus adjuvant therapy. The standard fractionation regimen in the United States is 1.8 to 2.0 Gy once daily, 5 days per week. The total dose of irradiation for definitive treatment is in the range of 70 to 80 Gy depending on the treatment schedule given and on the ability to shield normal tissue.

Common severe acute radiation toxicity includes epidermitis, mucositis, loss of taste, xerostomia, dysphagia, and hair loss. Dental evaluation and necessary extractions should be performed before radiation because dental extractions in a radiated mandible can lead to osteonecrosis. Dentulous patients should be given prophylactic fluoride. Patients receiving radiation are at high risk for tooth decay due to the xerostomia caused by injury to the salivary glands as well as mucosal damage.

Brachytherapy can be used as a definitive treatment for early-stage tumours or combined with external beam radiation in more advanced lesions in selected tumours (e.g., tongue, floor of mouth, tonsil, and nasopharynx) with excellent results. Brachytherapy is an option for recurrent cancers in the head and neck, particularly in previously irradiated patients[46].

**Chemotherapy:**

Until relatively recently, chemotherapy was used mainly for palliation of patients with locally recurrent or disseminated disease without proven survival advantage. Combination chemotherapy yields higher response rates but has increased toxicity with no proven survival advantage when compared with single agents. The choice of single-agent or combination chemotherapy depends on the patient's preference and performance status. Several Combination regimens have been developed to improve response rates. The combination of cisplatin and infusional 5-fluorouracil (5-FU) produces a 70% response rate and a 27% complete remission (CR) rate in chemotherapy-naive patients.

Platinum-based chemotherapeutic regimens and the single agent methotrexate are the most commonly used regimens for metastatic disease. Carboplatin may be slightly less active than cisplatin for head and neck squamous cancer, but carboplatin combinations with other chemotherapy agents are generally better tolerated than those with cisplatin. Carboplatin is preferred in patients at high risk for cisplatin toxicity, that is, those with renal dysfunction, neuropathy, or hearing loss.

Both docetaxel and paclitaxel have shown antitumor activity. Several dosing schedules for paclitaxel have been investigated. Three-hour infusions are probably the

best balance between theoretically optimum exposure and tolerable toxicity. Docetaxel is usually administered at doses of 60 to 100 mg per m2 every 3 to 4 weeks.

The role of chemotherapy has expanded significantly over the last decade because of the results of clinical trials incorporating chemotherapy in multimodality regimens for previously untreated disease.

**Induction Chemotherapy:**

Induction chemotherapy followed by definitive radiation therapy in patients responding to chemotherapy has been studied for organ preservation in patients with locally advanced cancers of the larynx and of the hypopharynx. No significant survival difference has been demonstrated for chemotherapy followed by radiotherapy compared to surgery followed by radiotherapy in these patients. For laryngeal cancer, concomitant cisplatin and radiation therapy leads to better local control. Presently, induction chemotherapy followed by radiation therapy can be considered standard only for patients with previously untreated locally advanced squamous cancers in the hypopharynx.

**Concomitant chemoradiation:**

The rationale for concomitant chemoradiation is based on experimental evidence of synergism between chemotherapy and radiation that is theoretically mediated by interference of chemotherapy with multiple intracellular radiation-induced stress-response pathways involved in apoptosis, proliferation, and DNA repair. The finding that certain chemotherapeutic agents (e.g., cisplatin, 5-FU, taxanes, and hydroxyurea) can induce radiosensitivity and increase log cell kill for radiation supports this treatment strategy. Cisplatin, the most extensively evaluated drug in recent large randomized trials, has the advantage of not having mucositis as toxicity, although as a radiation enhancer, it does increase radiation-induced mucositis.

**Adjuvant Chemotherapy:**

A large randomized study in resected patients with stage III or IV disease compared adjuvant radiation therapy with adjuvant chemotherapy followed by

radiation. This trial showed improved local control and overall survival rates approaching statistical

Significance for a subset of patients treated with chemotherapy who were at high risk for local recurrence. Patients with low-risk disease did not benefit from adjuvant chemotherapy.

Adjuvant concomitant cisplatin and radiation in patients at high risk for recurrence after surgery has been studied both in Europe and in the United States. Both studies found a possible benefit in disease-free or overall survival for patients receiving concomitant cisplatin and radiation[47].

**Prevention:**

1. Avoid unnecessary prolonged illumination, prevent the emergence of lip cancer

2. Avoid smoking and drinking.

3. Patients wearing dentures:

a. Found that tissue under dentures pain, inflammation, should seek immediate medical attention. Strive to achieve early detection of cancer, early diagnosis and early treatament, and insisted regularly checked.

4. Balanced diet

5. Do not drink and eat which will give irritation to oral tissues.

6. Unplug the residual tooth root and crown

7. Good wear dentures, does not stimulate tissue.

8. Develop good oral hygiene habits

9. Regular brushing. Pay attention to nutritional balance, timely treatment residual root.

**Four symptoms of oral cancer to be alert:**

If the mouth turns white, brown or black, it means a change in mucosal epithelial cells. Especially the oral mucosa becomes rough, thickened or showed induration, appeared oral leukoplakia, erythema, is likely to have cancerous.

**Unhealed ulcer:**

Oral ulcers lasting more than two weeks with burning sensation, pain and other symptoms should be altered

**Obvious pain:**

Early generally painless or only partial exception sense of friction, ulceration obvious pain, with further violations of the nerve tumor, can cause ear and throat pain.

**Lymph nodes:**

Multiple oral cancer to nearby lymph node metastasis neck, and sometimes the primary lesion is small, and even the symptoms are not obvious, but they are found in lymph node metastasis of cancer cells. Therefore, such a sudden neck lymph nodes, need to check the mouth.

**Vaccines:**

A number of infectious agents cause cancer. Hepatitis B and C are linked to liver cancer, some human papilloma virus (HPV) strains are linked to cervical and head and neck cancer, and Helicobacter pylori is associated with gastric cancer and lymphoma. Vaccines to protect against these agents may reduce the risk of their associated cancers. The hepatitis B vaccine is effective in preventing hepatitis and hepatomas due to chronic hepatitis B infection. Public health officials are encouraging widespread administration of the hepatitis B vaccine, especially in Asia, where the disease is epidemic. A four-valent HPV vaccine (Gardasil) is 100% effective at preventing infection. The vaccine is recommended for girls and women ages 9–26 years. Reduction in these HPV types could prevent >70% of the cervical cancers worldwide.

**PALLIATIVE TREATMENT IN TERMINAL STAGE**

- Pain relief with Morpia and Tramadol.
- Vomiting: correct dehydration and electrolyte balance[48].

## 3.3.PHARMACOLOGICAL REVIEW

Our great *Siddhars* explained many medicinal preparations to cure the life threatening cancer disease.

**Herbal Origin:**

**Compound herbal preparations**

**Pills**

- *Asuvagandhathi vadagam*

***Chooranam***

- *Garudakodi Chooranam*
- *Karanthai Chooranam*
- *Kukilathy Chooranam*
- *Megaroga Chooranam*
- *Vallathy Chooranam*

**Mineral and Metal Origin:**

*Siddhars* identified and worked on many metal and mineral preparations which had anti-cancer activity.

**Preparations:**

**Pills**

- *Mahakodasuzhi mathirai*

**Parpam**

- *Kariya parpam*[49]
- *Naga parpam*[50]
- *Rasa parpam*
- *Kandhaga poora parpam*[51]
- *Sootha parpam* [52]
- *Thalaga parpam*
- *Sanda rasa parpam*

***Chendooram***

- *Gowri chinthamani Chendooram*[53]
- *Gandhaga Chendooram*[51a]
- *Linga Chendooram* [54]
- *Kala mega narayana Chendooram*[55]
- *Navachara Chendooram*[56]
- *Karuvanga Chendooram* [56b]
- *Narayana Chendooram* [57]
- *Pavalavanga Chendooram*[58]
- *Sandamarutha Chendooram* [53a]
- *Astabairava Chendooram*
- *Naga Chendooram* [59]
- *Rasa Chendooram*
- *Thambira Chendooram* [55a]

**Pathangam**

- *Guru pathangam* [60]
- *Veera rasa pathangam*
- *Putrupathangam* [61]

**Thylam**

- *Singi thylam*[62]
- *Pupudhakkar mega thylam*
- *Pachai thylam*[62a]
- *Sengathari thylam* [63]
- *Vippuruthiennai* [63a]
- *Mega rasangaennai* [63b]
- *Chinthamaniennai*
- *Sengottai thylam*
- *Visharajanga thylam*
- *Meganathiennai*
- *Magasanthanathy thylam*[64]

**Nei**

- *Kukkilnei* [65]
- *Thengainei*
- *Vallarainei*

***Mezhugu***

- *Korosanai mezhugu*
- *Gandhaga mezhugu* [51b]
- *Kanagalinga mezhugu*
- *Guru sanjeevi mezhugu*
- *Valai rasa mezhugu*
- *Veera mezhugu* [66]

**Others**

- *Madhusmeegi rasayanam*
- *Kulirntha pachai*
- *Veelai seelai*
- *Rana pugai* [62b]

- *Chitravallathi lehiyam.*

**Siddha drugs for oral cancer:**

**Pills**

- *Chithramoola kuligai*

***Chooranam***

- *Karanthai chooranam*

***Rasayanam***

- *Parangichakkai rasayanam*

***Parpam***

- *Thambiraparpam*
- *Rasa parpam*[67]
- *Gandhagaparpam*
- *Karuvangaparpam*
- *Naga parpam* [68]

***Chendhooram***

- *Kalamega narayana chendhooram*[69]
- *Pancha padanachendhooram*[70]
- *Swarnapushpa rasa chendhooram*
- *Muthuchendhooram*
- *Sadakshara chendhooram*

***Nei***

- *Chitramoolanei* [71]

***Ennai***

- *Perungayaennai*

- *Gandhagathylam*

***Mezhugu***

- *Rasagandhimezhugu*[72]
- *Gandhagamezhugu* [73]
- *Korosanaimezhugu*
- *Markandeya mezhugu*
- *Vaan mezhugu*
- *Nandhi mai*

***Kattu***

- *Poorakattu*

***Padhangam***

- *Linga padhangam*
- *Bhramasthiram* [74]
- *Guru padhangam* [75]

**Anticancer Drugs- Modern Aspect:**

The drugs which prevent neoplasm are known as anticancer drugs. They also called antineoplastic drugs.They may be divided into two classes

- Cycle specific

Cycle specific drugs act only at specific points of the cell's duplication cycle, such as anaphase or metaphase

- Non- cycle specific

Drugs may act any point in the cell cycle, In order to gain maximum effect, anti-neoplastic drugs are commonly used in combinations.

**Table no:2 Classification of Anti -Cancer Drugs [76]:**

| | |
|---|---|
| 1.Alkylating agents<br>Nitrogen mustards<br>Ethylenimines<br>Alkyl sulfonate<br>Nitrosoureas<br>Triazine<br>Methyl hydrazine | Mechlorethamine, cyclophosphamide, ifosfamide, chlorambucil, melphalan, bendamustine<br>Thio-Tepa, Altretamine<br>Busulfan<br>Carmustine, streptozocin<br>Dacarbazine, temozolomide<br>Procarbazine |
| Antimetabolites<br>Folate antagonist<br>Purine analogues<br>Pyrimidine analogues | Methotrexate (amethopterin), pemetrexed<br>6-Mercaptopurine, thioguanine, pentostatin, fludarabin, cladribine.<br>5-Fluorouracil, floxuridine, capecitabine, cytarabine (cytosine arabinoside) gemcitabine |
| Natural and semisynthetic products<br>Antibiotics<br>Epipodophyllotoxins<br>Camptothecins<br>Taxanes<br>Vinca alkaloids | Actinomycin-D (Dactinomycin), daunorubicin, doxorubicin, bleomycin, mitomycin-C, mithramycin<br>Etoposide, teniposide<br>Topotecan, irinotecan<br>Paclitaxel, docetaxel<br>Vincristine, vinblastine, vinorelbine |
| Miscellaneous | Hydroxyurea, cisplatin, I-asparaginase, imatinib, bortezomib, thalidomide, monoclonal antibodies. |
| Hormones and their antagonists | Glucocorticoids, androgens, antiandrogens, oestrogens, antioestrogens, progestins, aromatase, inhibitors. |
| Biological response modifiers | Interferon alpha, interleukin 2, amifostine, haematopoietic growth factors |

**Interactions:**

Anticancer drugs may interact with a number of other medicines. When this happens, the effects of one or both of the drugs may change or the risk of side effects may be greater

**Table no:3 ANTI-CANCER DRUGS:**

| DRUGS | MOA | USES |
|---|---|---|
| Cyclophosphamide | Forms reactive derivates, alkylates DNA and important groupscytotoxicity | NHL CLL, breast, ovarian cancer, soft tissues sarcoma, Wilms tumour, Rhabdomyosarcoma. |
| Busulfan | Same as above | CML |
| Methotrexate | Folate antagonist-MTX, polyglumates decreases DHFR-inhibits protein synthesis. | Choriocarcinoma, NHL, breast, bladder, head, and neck cancer, osteogenic sarcoma. |
| Mercaptopurine | Purine analog-incorporated into DNA and RNA- breaks into DNA, inhibits DNA synthesis. | AML |
| 5-Fluorouracil | Pyramidine analog-incorporated into DNA and RNA- inhibits DNA synthesis, inhibits TS. | Colourectal, anal, hepatocellular, gastric, ovaries, head, and neck cancers. |
| Actinomycin-D | Inhibits DNA dependent RNA synthesis. | Wilmstumour, Ewings tumour rhabdomyo sarcoma, chorio carcinoma, kaposis and soft tissue sarcoma, immuno suppressant. |

| DRUGS | MOA | USES |
|---|---|---|
| Bleomyocin | Bind iron, generates free radicals-breaks into DNA | Testicular tumours, Head and neck cancer, HL and NHL |
| Daunorubicin & Doxorubicin | Bind DNA and inhibits topoisomerase. | Testicular tumours. Head and neck cancer. |
| Etoposide | Inhibits topoisomerase II | Lung and Gastric cancer. |
| Topotecan | Inhibits topoisomerase I | Lung and Ovarian cancer. |
| Vinblastine | Inhibits mitosis | Breast cancer, Kaposi's sarcoma |
| Vincristine | Inhibits mitosis | Neuroblastoma |
| Cisplatin | Active form inhibits DNA synthesis. | Lung, breast, bladder, testis, ovarian, head and neck cancers. |

**Common adverse effects of anti-cancer drugs:**

Because antineoplastic agents do not target specific cell type, they have a number of common adverse side effects.

- Hair loss is common due to the effects on hair follicles
- Anaemia
- Immune system impairment
- Clotting problem caused by destruction of the blood forming organs, leading to reduction in the number of red cells, white cells and platelets.
- Bone marrow depression
- GIT-stomatitis, glossitis, esophagitis.
- Reduced spermatogenesis in men and amenorrhoea in women
- Nausea and vomiting are immediate side effects
- Hyperuricaemia (increased plasma uric acid levels) leads to renal failure
- Carcinogenicity (cause secondary cancer).

**SCREENING METHODS:**

The pharmacological screening of plants, minerals and animals is an essential mean for the invention of new, harmless and effective drugs. Over 50,000 plants have therapeutic virtues in the world, and around 80% of human use medicines based on plants and salts at least once in their life, Medicinal plants and mineral have diversified chemical constituents which are important for the discovery of new active molecules against many types of cancer.

Active compounds from many medicinal plants and minerals with effective cytotoxic properties were developed in to anti-cancer drugs.

Nowadays it has become mandatory to monitor the quality of life of patients while in treatment of cancer. It is healthy aware that the quality of life of cancer patients treated with chemotherapeutic drugs are very much affected even long time after withdrawal of drugs.

Therefore, the challenging task at this moment is to identify the quick and novel methods that can identify and develop molecules, which can be of therapeutic value in human cancers.

Cancer is one of the thrust area for which effective drugs at comfortable prices are not available as yet probably due to lack in understanding the cancer Patho physiology. For such a dreadful disease anti-cancer drugs have been developed from a variety of sources ranging from natural products (plants and microbes) to synthetic molecules. One of the cause of treatment failure is the development of resistance to anticancer agents.

The widely used drugs which are called as cancer chemotherapeutic agents have many side effects such as bone marrow suppression, alopecia, nausea and vomiting.

This necessitates screening of a large number of compounds .For this purpose both in-vitro and in-vivo models are employed for systematic screening of an anticancer drugs[77].

**INVITRO METHODS:**

In studies in vitro cytotoxicity on cell line, various cell staining methods are used in order to indirectly estimate the number of viable cells present after treatment. An ideal test in assessing cell proliferation and cytotoxicity should have main feature in vitro: be simple, fast, efficient, economical, reproducible, sensitive, safe, and effective as far viable cell population and do not show interference with to evaluate the compound. In-vitro testing is a potential chemotherapeutic agent.

**ADVANTAGES[78]:**

- Easier to manage
- Less time consuming
- Small quantities and large number of compounds can be tested.
- Reduce the usage of animals
- Testing the ability of the compound to kill the cells by taking the advantage of various properties of cell
- Able to process the large number of compounds quickly with minimum quantity.
- Range of concentrations used is comparable to that expected for in vivo studies.

**DISADVANTAGES:**

- Pharmacokinetics in determining drug effects cannot be evaluated.
- Growing Solid tumour is poor compared to in-vivo method.
- Difficulty in maintaining the culture
- Show negative results for the compounds which gets activated after metabolism and vice versa
- Impossible to ascertain the pharmacokinetics.

**How to culture cell line**

- Tumor cell line derived from several cancer types.
- Adaptable to a suitable growth medium.
- Show reproducible profile for growth and drug sensitivity.

- The lines were prepared and preserved using regents such as DMSO during freezing.
- Thawing- bringing the freezed ampoule to room temperature by slow agitation.

**Ideal Characteristics of an In vitro Screening Method:**

An ideal in vitro screening method should be simple, economical, reproducible, rapid and sensitive. The assay should be applicable to large number of tumour types and test compounds. The choice of the cell lines should be representative of clinical situation as close as possible. The range of drug concentrations used in vitro should be comparable to that expected for in vivo treatment. The assay should be able to process a large number of samples quickly and in an automated fashion. Data acquisition should be simple, easily interpreted and applied. The goal of a screening assay is to test the ability of a compound to kill cells, at the same time, the assay should be able to discriminate between replicating cells and non-replicating cells (quiescent cells that are dead or dying apoptosis).

**Table :4 Different assays take disadvantage of various properties of cells as mentioned below:**

| S.NO | CELL PROPERTIES | ASSAYS |
|---|---|---|
| **1** | Enzymatic properties | Tetrazolium salt assay(MTT) |
| **2** | Protein content/synthesis | Sulphorhodamine B assay |
| **3** | DNA content/synthesis | H-Thymidine uptake Newer fluorescent analogues with flow cytometry Clonogenic assay |
| **4** | Membrane integrity | Dye exclusion tests |
| **5** | Clonogenic properties | Clonogenic assay |
| **6** | Cell division | Cell counting assaY |

**Tetrazolium Salt Assay (Microculture Tetrazolium Test or MTT):**

MTT assay is an international accepted in vitro method for anticancer drug screening. Though viable cells can be measured using several methods other staining procedures also used these which suffer from drawbacks that the require washing steps thereby increasing processing time and sample variation. The multi well plate scanning spectrophotometers can quickly measures large number of samples with high degree precision and accuracy. Ideally, a colourimetric assay for living cells utilize a colourless substrate that is modified to a coloured product by any living cells, but not by non-viable or dead cells or culture medium. However, MTT assay utilizes a colour reaction as a measure of viable cells. The assay is dependent on the cellular reduction of 3-(45dimethylthaizol-2-yl) -25-diphenylltetrazolium bromide, a tetrazolium salt to a blue formazan product by the mitochondrial dehydrogenase of viable cells/ metabolically active cells. The intensity of blue coloured formanzan produced is directly proportional to cell viability.

The cells from a particular cell line when in log phase of growth are trypsinized, counted in a hemocytometer and adjusted to appropriate density in a suitable medium and then inoculated in different multi well plates (usually 96-well plates). The cells are treated with various concentrations (in replicates) of drugs for specific duration (usually 1 to 4 days), after which MTT dye is added in each well and plates are incubated at 37° for 4 h in a co2 incubator. The plates are taken out of incubator, dark blue coloured formazan crystals are thourougly dissolved in isopropanol / DMSO at a room temperature. The plates are then read on an ELISA reader at 570 nm. The percent cell viability with respect to control is calculated using the formula:

$$\% \text{ Cell viability} = \frac{\text{OD of treated cells}}{\text{OD of control cells}} \times 100$$

This assay has been successfully used by us. The DMSO as a solvent rapidly solubilizes the serum as well as formazan and use of spectrophotometric grade DMSO gives stable "background" absorbance levels. Other solvents like isopropanol, propanol,

hexane and dimethyl formaldehyde, though used, do not solubilize serum at concentrations exceeding 0.0625 percent.

The advantage of this assay is that, it can be run on microtiter dishes on hundreds of cell samples at one time so that the various drug concentrations can be used to get an idea of the dose response relationship for each drug tested. As a result, this assay can be adopted for the determination of IC50 of drugs (concentration of drug required to inhabit 50% cell growth).

Further this assay is relatively simple and therefore easy to perform. It can be used for both adherent and suspension cell lines. This method is cheap, requires low number of cells, manageable and a large number of drugs can be quickly screened for anti-proliferative activity. However, the assay suffers from a drawback of giving false results due to the inclusion of cells that might metabolically be active due to cells are not capable of dividing (non-replicating). In addition, drugs whose mechanism of section might spare mitochondria may not yield positive results in this assay, especially for short incubation times. Also, use of DMSO warrants safe handling by laboratory personnel.

**Sulphorhodamine B Assay**:

The Sulphorhodamine B (SRB) assay measures whole-culture protein content, it should be proportional to the number of cell. Cell cultures are stained with a protein staining dye, Sulphorhodamine B. It is a bright pink dye, which binds to basic amino acids of cells.Un bound dye is then removed by washing with acetic acid, and protein-bound dye extracted using unbuffered. Trisbase for determination of optical density in a computer-interfaced, 96-well microtiter plate reader. Since dead cells either lyse or are lost during the procedure, the amount of SRB binding is propotional to the number of live cells left in a culture after drug exposure. This assay can be used to measure the cellular protein content of both adherent and suspension cultures. Screening capacity, reproducibility and quality control all appear to be enhanced in this assay relative to the tetrazolium salt assays. The assay is more cumbersome and time consuming compared to the MTT assay. Non-replicating and dead cells might contribute to the total protein and interfere with the results.

**H-thymidine Update Assay:**

In this assay tumor cell suspensions are exposed to the drug continuously for 5 days, after which a radio-labeled precursor (3H-thymidine) is added during the final 48 hours of the assay to label proliferating cells. The replicating cells will incorporate [3H]- thymidine into their DNA, which can then be determined either by autoradiography or by liquid scintillation counting, Auto radiographic determination of the [3H]-thymidine, though, Is time- consuming but it provides information on tumor growth kinetics. This can generate DNA histograms, which can provide information on the ploidy status of the cells. This assay looks at cells, which have actively replicating DNA and hence are viable, Non replicating or dead cells will not be counted in this case. The assay can be used for both adherent and suspension cell lines. The assay is rapid, relatively inexpensive, and feasible in the majority of tumor types. However, it will not differentiate between malignant and non-malignant cells and might lead to false-negative predictions if lethally damaged cells undergo a final division.

**Fluorescence:**

Fluorescent dyes may be used in conjunction with microscopic evaluation methods as an in vitro chemo sensitivity assay. Cells are exposed to fluorescent-labeled precursors after drug-exposure. The replicating cells will incorporate labeled precursor into their DNA and the resulting fluorescence is then measured by flow cytometry. This assay also looks at actively replicating cells and hence dead or non-replicating cells are not counted. In addition, the assay does not involve the use of radioactivity and is useful for adherent and suspension cell-lines. Also using flow cytometry it is possible to determine that in what phase of the cell cycle the cells are. The quantitation of apoptotic cells is also possible. However this, assay requires the data to be analyzed by an expensive and sophisticated fluorescence activated cell-sorter (FACS) instrument. Because of technical difficulties in applying flow cytometry to primary tumor specimens, data on the predictive value for clinical response for this assay are too scarce to permit definitive conclusions.

**Dye Exclusion Tests:**

Early attempts to use exclusion of vital dyes like trypan blue, eosin, or nigrosin to predict chemo sensitivity were unsuccessful. These assays relied on the structural integrity of the cells .Dead cells would have lost membrane integrity and hence would take up vital dyes like trypan blue. This method was mainly used because of its technical simplicity No prospective trials of these assays have yet been performed, however, to demonstrate their ability to predict as lack response or lack of response. This DISC assay is drug sensitive assay, which relies on structural integrity of the cells. In this assay, cells are incubated with drugs for 4 days. Dead cells are stained in suspension with fast green dye with or without nigrosin. The specimen is centrifuged and disks of cells are collected in the microscopic slides. Live cells are then stained with hematoxylin-eosin. As control duck erythrocytes are used. The end point of the study is the morphologic identification of tumor-cell cytotoxicity compared with the internal control standard of duck erythrocytes. The DISC assay measures cell kill in both dividing and non dividing and tumor cell population.

**Clonogenic Assays:**

A concern in the use of anti-proliferative assays is that they measure growth inhibition rather than cell killing. This is particularly important for drugs that act by arresting cells at check points in the cells cycle. Checkpoint arrest is a survival response of cells that allows repair of DNA damage and is therefore not directly related to the induction of cell death. Thus, cells that act by arresting cells at checkpoints may show lower IC50 but increased survival. Clonogenic survival assays on the other hand, measure loss of tumor cell reproductive viability(the ability of a single cell to form colonies).it is the most direct method of measuring cytotoxic activity of a drug, In clonogenic assays single-cell suspension are prepared from tumor biopsies and exposed to anticancer agents to be tested . Cells are then rinsed and plated in a semisolid medium (agar or methy1 cellulose),a medium that precludes proliferation of nonmalignant cells in the specimen.15 After 14 to 28 days, some cells will have undergone several divisions and will have formed tumor colonies, which can be quantified in a visual or semi-automated fashion. Non-replicating and dead cells are not coated in this case. The number of colonies from the treated cells is compared with the number of colonies from

the untreated control cells and the fraction of control growth provides an index of drug activity. Traditional clonogenic systems suffer from a number of significant technical problems like long incubation time(at least 14 days)before results can be made available to the clinician. The assay is labor-intensive, costly, and cannot be used for suspension cell-lines.

**Cell Counting Assay:**

Cells are cultured in the presence of drug for 2-5 culture-doubling times, after which the cell number is estimated using a hemocytometer or a cell counter. The assay is easy to perform, rapid and can be used for both adherent and suspension cell lines. However, dead and non-replicating cells can be counted in this assay by the cell counter. The IC50 values can be calculated in all the above assays.

**Assay for Energy metabolism and autophagy**

- FAD assay
- ATP assay
- Lysosome detection
- Mitochondrial membrane potential assay
- Reactive oxygen species test

**For nuclear signaling, DNA damage and cell proliferation**

- $P^{53}$assay
- Topoisomerase II assay
- $P^{21}$assay
- Cell proliferation assay
- Mdm2 assay

**For inflammation, angiogenesis and metastasis**

- Cytokine and chemokine assay
- STAT 1,2,3,6 assay
- COX-2 activity assay
- LDL uptake assay

**For apoptosis, pyroptosis and necrosis**

- Caspase 1 assay
- Bax assay
- Cytolysis assay

**For cancer signaling pathway and phenotype**

- ERK assay
- c- AMP assay
- c- Jun test [79]

**Cell lines for cancer**

There are plenty of cell lines available for research purpose. Only very few are listed [80].

**Table no: 5 Cancer cell lines**

| S.no | Cell name | Tissue | Species |
|---|---|---|---|
| 1 | UM-UC | Bladder | Human |
| 2 | FM3A | Breast | Mouse |
| 3 | C 170 | Colon | Human |
| 4 | SHP 77 | Lung | Human |
| 5 | RAG | Kidney | Mouse |
| 6 | HF 1 | Liver | Rat |
| 7 | MEWO | Skin | Human |
| 8 | TT | Thyroid | Human |
| 9 | OV | Ovary | Human |
| 10 | C 6 | Neural(Glialtumor) | Rat |

**IN VIVO MODELS:**

In vivo models are advantageous over in vitro models in the sense that they can detect host-mediated activity, are relatively predictable and estimate therapeutic ratio. However, as compared with in vitro systems, their sensitivity is low, are costly, time consuming and large number of samples cannot be handled and are difficult to manage. After all, animal models are used both toxicological studies and for detecting preclinical anticancer efficacy. They are able to detect agents irrespective of their mechanism of action. The drugs with high degree of efficacy and broad spectrum of activity in animal models are usually expected to be effective in clinical cancer, however, there are exceptions also which could be due to metabolic differences and heterogeneity of cancer cells between human and rodents. Despite these differences animal models are widely used to support the results obtained from in vitro studies. The most promising candidate compound is tested in more than one animal model. Dose response relationship, combined effect of drugs, modes of their anticancer action and organ specificity are established. Varied drug dosage forms, doses and animal strains and animals of a particular age group may be used. The selected animal models should be representative of high incidence of human cancers. The in vivo anticancer drug screening methods are described under the following headings:

A. Chemically induced tumor models

B. Models involving cell line/tumor pieces implantation.

**Chemically Induced Tumor Models**:

Chemical carcinogens are well-known to account for about 80% of all cancers and are used to induce cancer in animal models. Carcinogens require metabolic activation before inducing carcinogenesis. The epidemiological studies indicate that human carcinogenesis occurs through multiple steps in the same way as in mouse skin. The concept of multistep carcinogenesis was first of all developed in rodent skin models in 1940s and applies to cancers to many species and cell types. Experimental carcinogenesis involves following three steps:

1. Initiation is due to exposure to carcinogens transforming the normal cell to a cancer cell. Many animal species develop cancers spontaneously and are valuable for understanding the biology of sporadic cancer development in humans. The major use of spontaneous cancer models is to compare the biology with human; these animals are increasingly valuable for cross-comparison of response or resistance to the same clinical agents used for patients.

2. Promotion is due to the triggering of uncontrolled growth of the transformed cell.

3. Malignant conversion is caused due to unlodging of cancer cells from the original site its transportated by circulation and the establishment of secondary tumors in the body.

The exact sequence of cellular, biochemical and molecular genetic events may differ between tissues and species, the overall concept seems to be directly applicable to clinical cancer and thus in future multistage mouse skin carcinogenesis model will be of immense utility for further understanding the mechanisms of epithelial carcinomas in human beings.

The experiment is well designed; dose of the carcinogen as well as drug treatment schedule is standardized by conducting pilot studies. This helps in accurate evaluation of the test compound[81].

**DMBA-induced Mouse Skin Papillomas:**

This is a classical two-stage experimental carcinogenesis model. Mouse skin is generally most sensitive to epidermal carcinogenesis, Rats, hamsters and rabbits are less sensitive and guinea pig is very resistant 2. SENCAR mice are highly sensitive to DMBA induced skin tumors .Swiss albino mice are relatively less susceptible to tumor induction. DMBA acts as an initiator and 12-o-tetradecanyl-phorbol-13-acetate(TPA) is used as a promotor to induce skin papillomas and squamous cell carcinomas. Mice are topically applied a single dose of 2.5µg of TPA in 0.2 ml acetone twice weekly on the same site starting one week after DMBA application. Papillomas begin to appear after 6 to 7 weeks of application of TPA.

Weekly observations are made to monitor tumor development till the experiment terminates after 18 weeks. Percent tumor incidence and multiplicity of treatment group is compared with DMBA control group. Drug under test can be administrated either topically or by oral route. The tumor incidence in this model is usually about 100% in DMBA controls. In various laboratories, however, repeated topical application of DMBA alone has also been shown to induce carcinogenesis

**3. The development of papillomas in DMBA treated swiss albino mouse (24th week).**

**MNU-induced Tracheal Squamous Cell Carcinoma in Hamster:**

In this model, 5% solution of MNU in the normal saline is administrated once a week for 15 weeks using specially designed catheter, which exposes a defined area of the trachea of male Syrian golden hamsters to the carcinogen.

Fifteen weeks MNU administration produces tumors in 40-50% animals within 6 months. Test drug efficacy is measured as percentage reduction of tumor incidence compared with carcinogen control.

**DMBA-induced Oral Cancer in Hamster:**

Oral cancer can be induced in a male Syrian hamsters painting right buccal mucosa, 3 times/ week for 16 weeks with 0.5% solution of DMBA in liquid paraffin (approximately 10 µl containing 100 µg). Tumour size, number and tumour burden of drug treated animals can be compared with that of control animals at the termination of experiment.

**Animal models:**

**1.Mouse cancer models**

a. GEM-Genetically Engineered mouse Models
b. Inbred mice (systematic sibling mating)
c. Transplantation models
   Allograft models (syngeneic tumour tissues derived from same genetic mouse)

Xenograft models (actual human cancer cells or solid tumours are transplanted into host mouse)

d. Carcinogen induced and spontaneous models

1. Digestive system cancer induced by polycyclic aromatic

2. Chemically cancer induced by Cadmium and Arsenic

3. Radiation-skin cancer by ultraviolet radiation, leukemic changes by ionizing radiation.

**2. Rat cancer models:**

**a.Genetically altered rats**

1. Treat embryos with DNA damage causing chemical mutagen, Frequently N-ethyl-N-nitrosurea (ENU) is used.

2. Insertion of mutagenesis strategies (Retro viruses)

3. Transgenic strategies (pronuclear injection of DNA)-quickly developed and more effective models.

**b. Inbred rats.**

**3. Other laboratory animal models**

a. Hamster

b. Rabbits

c. Zebrafish

**4. Other animal models**

a. Dogs

b. Cats

c. Goats

d. Horses

e. Pigs.

## 3.4. PHARMACEUTICAL REVIEW

***Chendooram:***

**Definition:**

*Chendooram* is a category of medicines made from metals or minerals (arsenicals or mercurial's or salts) by grinding them with specified juices or distillates or extractives and subjecting them to a process of sublimation or calcinations or burning or frying or exposing to insolation till the characteristic reddening of the product takes place. The *Chendooram* are said to retain their potency for 75 years

**Method of preparation:**

Usually two method of preparation are adopted in their processing, with some exceptions and variants. Such as:

1. Sublimation by the sand – bath process

2. Calcination.

**1. Sublimation by the sand - bath process (*Kuppi Erippu)*:**

If the *Chendooram* has Sulphur and Mercury as its components, sulphur is ground to a fine powder in the mortar and grinding should be continued with the addition of the given quantity of Mercury, till a black impalpable mobile powder is obtained. Only after this, ,other ingredients are to be added.

In the conventional set up of the sand –bath sublimation contrivance, a heat resistant glass flask with a long neck is used as the container for the drug ingredients. Ceramic ware is also been in used. Before being put to use, these container are wound around with clay smeared cloth ribbons so as to give seven superimposed layers, leaving open the mouth of the flask. The flask thus encased should be kept for perfect drying of the covering.

It has been found in recent times that one could make use of the enameled iron bowls instead of glass flasks. When using enameled iron bowls, two identical bowls of appropriate dimensions and capacity should be selected and checked for neat contact of rims when juxtaposed. Then small holes should be punched along the margins so that the two bowls could be fastened with a bonding wire (metallic). Then a perforation is made in the center of the bottom of one of the bowls. Having prepared the bowls thus, they should be secured and bound by pasting the binding wire through the marginal holes. This would produce a capsule with a top orifice. Clay smeared cloth tape is wound around as would be done for the glass flask, leaving the central opening uncovered. This opening is the one through which the reaction going on inside is inspected by inserting a probe.

The sand – bath is set up by taking a wide earthen trough and spreading fine gravel or coarse sand at the bottom to a depth of two centimeters.

The capsule into which the drug ingredients are put is placed on the gravel or sand and is properly cantered. Then the sides packed with sands, leaving the top two centimeters unpacked and exposing the capsule. When using glass flasks, the neck should be just out of the sand. This setup is placed on the oven and heat is applied, by burning fire wood.

In the application of heat, there gradations are recognized. These three stages, mild, moderate and intense are best understood and mastered with some experience.

It is said that, if the flames are convergent and resemble a single tongue of flame as in a lamp, it is mild fire (*Deepakkini*). If several such tongues of flame lick the vessel and diverge like the flower of lotus, it is moderate (*Kamalakkini*). If the multiple tongues of flame fill the oven and enrich the sand bath. It is the intense stage of fire (*Katakkini*).

These stages of fire should be manipulated and followed as prescribed in the method of preparation. In general, the heating is spread over three continuous days. In such cases, mild, moderate and intense stages are maintained for 24 hours each, in that order of succession.

According to the composition and amount of sulphur in the preparation, the mixture of drugs placed in the capsule will start melting sooner or later. Sulphur starts escaping first in the form of yellow vapour through the opening. Later it will start burning sending out a jet of blue flame. Just when the blue flame goes out, it a long probe of steel wire is inserted into the orifice and drawn out the portion that enters the container will show a whitish coating. If the sulphur is still present and not totally burnt out, the probe will have a black sticky coat, when there is no blackening of the probe and when whitish coat indicating should be closed and heating continued for one or two hours and then the heat withdrawn and the setup is allowed to cool by itself.

When the setup has cooled down, the capsule containing the medicine is taken out and the clay tape winding cut out. The material that has sublimed in upper bowl is gently tapped with suitable beater or lifted with a spatula. The sublimate collected should be finely ground in a mortar.

If the glass flasks have been used, the flask is carefully broken, open to collect the medicine that has sublimed in around the neck.

**2. Calcination (*Pudam)*:**

The powder is ground in a *Kalvam* with specified fluids for a specified time. The paste is made into small discs and dried. They are put in earthen saucers (*mann agal*) covered with another and the edge well sealed with mud cloth. It is allowed to dry. The cups are placed in the middle of cow – dung cakes and burnt. For *Pudams,* generally pits of various depths and circumferences are made in the ground. Half of the pit is covered with cow – dung cakes. The earthen cups are placed and it is covered again with cow-dung cakes. The fire is put in the middle of the heap on all the four sides so that there would be uniform heat from all the sides.

All the metals and other ingredients are taken after the usual purification. In specified cases, specific purification (*Suddhi*) is mentioned; otherwise, it is to be taken as general method of purification for the drug as mentioned in Materia- Medica books.

**Other method of preparations:**

1. Prepared without heating (*Araippu Chendooram)*

2. Prepared by open heating (*Erippu or Varuppu Chendooram)*

3. Prepared by applying heat in the range close to 100◦c (*LaguPuda Chendooram).*

**Specifications for *Chendooram***

1. *Chendooram* is red in nature, well fine in particle size and tasteless.

2. With suitable adjuvant they possess therapeutic values.

3. They are said to retain their potency for 75years[82].

**Table no:6 ANALYTICAL SPECIFICATIONS OF *CHENDHOORAM*** [83]

| S.no | Test |
|---|---|
| 1. | Description-colour, odour |
| 2. | Identification-chemical |
| 3. | Particle size-200 to 300 |
| 4. | Loss on drying at 105ºc |
| 5. | Total ash |
| 6. | Acid-insoluble ash |
| 7. | Water soluble ash |
| 8. | Assay of element (s) |
| 9. | Siddha specifications |
| 10. | Lusterless |
| 11. | Fine enough to enter the cervices of finger |
| 12. | Floats on water |
| 13. | Smokeless |
| 14. | Tasteless |
| 15. | Irreversible |

## 3.5 LATERAL REVIEW

**Potassium nitrate (*Vediuppu)***

Medical Education Online reports that "potassium supplements, 40-80 mEq/day, lower blood pressure, an effect that is largely lost in patients who are also on a low sodium diet." The scientific community continues to be uncertain about the way in which potassium is related to high blood pressure and vascular diseases. Potassium and blood pressure appear to be inter-related through changes in sodium excretion[84].

**Aluminum sulphate ( *Padikaram* (alum):**

Alum with its concentrations of (5 and 10) gm/100 ml sterilized by a Millipore filter and gauze was affected against most bacterial isolates while alum in concentration 2.5 gm/ 100 ml not showed any inhibited affect against all selective bacterial isolates[85].

**Copper sulphate (*Thurusu)*:**

In the Microbial analysis, The drug, *'Thurusu chendooram'* in shows sensitive to all the organisms tested. This drug has *which copper sulphate as a main drug* moderate sensitivity to *E.Coli, Klebsiella, Styphylococcus aureus, Pseudomonas aeruginosa*, *Candida albicans* and highly sensitive to *Proteus* and *Streptococcus pneumoniae*. This drug has been indicated to *Gunmam* (A.P.D), *Peruvayiru* (Ascities) and diseases due to the derangement of *Vatha, Pitha* and *Kapha*[86].

**Sodium borate (*Vengaram):***

Borax shows a potent anti-inflammatory and healing properties. Hence, it has been used as a treatment in chronic tonsillitis in the form of a gargle. To ensure scientific validity of the efficacy, a comparative study is conducted between Aspirin tablet and borax which was statistically analyzed. In the study borax showed a significant relief from symptoms that were statistically significant [87].

**Ammonium chloride (*Navachaaram)* :**

- Ammonium chloride shows a diuretic effect, diuretics are able to reduce the blood pressure which plays important role in treatment of angina. Diuretic drugs influences the reabsorption of ionic sodium at tubular levels. Thus it reduces the blood pressure, cardiac filling, and ventricular stroke volume etc.

- Ammonium chloride which is a ingredients of *Veera Mezhugu* possess both an antioxidant and anticancer potentials justifying scientifically its administration in cancer patients by Siddha practioners[88].

**Mercury (*Rasam)*:**

- The drug *Arkashara Rasa* showed potent activity against pancreatic cancer cells (MIA-PaCa-2). LDH activity confirmed that AR was active against **Pancreatic Cancer** cells.
- The incineration process of the Mercury and Sulphur macro particles are became very smaller and this may possible for devoid of toxicity and more potent in **Anticancer** therapeutic.
- The results acquired from the *in-vitro* studies achieved via the ***HeLa* cell** lines reveals the unique Siddha medicine *Gowri Chinthamani Chendhooram* (contains Mercury and Sulphur) have a potent **Anticancer activity[89].**

**Cinnabar (red Mercury (II) Sulfide (HgS) (*Lingam)*:**

The study shows a *Mupoora chendurum* in which cinnabar is one of the ingredients was prepared as per the Siddha textual references, with Sophisticated tests reveals the absence of heavy metals like Arsenic, Lead, Cadmium and Copper. Mercury was within the permissible limit. The Particle size of the drug was 83.3 nm. In spectra by visual gratitude, there are no significant difference in the characteristic absorption bands but the intensity of certain wavelength do differ from each other especially at the fingerprint region (3584–1035 cm-1). This report is a fingerprint for future references in analysis of *Mupoora chendurum*, the drug has antibacterial activity against *Bacillus, Streptococcus and Vibrio*[90].

**Yellow Orpiment (*Thaalagam*):**

- In the Microbial analysis, the *'Thalaga parpam'* was sensitive for only one organism, in which Orpiment is one of the ingredients shows a Streptococcus pneumoniae with 18 mm of inhibition.
- Low doses of arsenic trioxide can induce complete remissions in patients with APL who have relapsed. The clinical response is associated with incomplete cyto differentiation and the induction of apoptosis with caspase activation in leukemic cells[91].

**Sulphur (*Gandhagam)*:**

- The growth inhibitory and apoptosis-related effects of a newly developed highly purified sulphur (HPS) on immortalized human oral keratinocytes (IHOKs) and on oral cancer cells representing two stages of oral cancer (HN4, HN12) based on a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, Western blotting, cell cycle analysis, and nuclear staining.
- The both diol-containing compounds, 2a and 3, were the most cytotoxic of the sulfide series against V-79 cells in vitro (IC(90) = 2.1 micro M and 1.9 micro M, respectively). A preliminary **anticancer s**creening against **P388 leukemia** showed that 2a is highly active in vivo as well.
- Ally sulphur compounds from garlic are reported to reduce the incidence of Breast, Colon, Skin, Uterine and Lung cancers and to depress proliferation of tumour cells[92].

**Realger (α-$As_4S_4$) Arsenic Sulphide (*Manosilai)*:**

Arsenic disulfide, a major effective component of realgar, has been investigated for its anti-cancer potential and shown to have therapeutic efficacies in hematological and some solid tumors. However, its effect against breast cancer is rarely reported. The study shows a anti-cancer effects of $As_2S_2$ in human breast cancer cell lines MCF-7 and MDA-MB-231. $As_2S_2$ significantly inhibited cell viabilities, induced apoptosis, and led to cell cycle arrest in both cell lines with a dose- and time-dependent manner. $As_2S_2$ upregulated pro-apoptotic proteins like p53 and PARP in MCF-7 cells. Besides, $As_2S_2$ downregulated anti-apoptotic proteins like Bcl-2 and Mcl-1, as well as cell cycle-related proteins cyclin A2 and cyclin D1 in both cell lines. Of note, the expression level of cyclin B1 was downregulated in MCF-7 cells, whereas, upregulated in MDA-MB-231 cells. Moreover, $As_2S_2$ significantly inhibited the pro-survival signals in PI3K/Akt pathway in both cell lines. These results provide fundamental insights into the clinical application of $As_2S_2$ for treatment of patients with breast cancer[93].

# 4. MATERIALS AND METHODS:

## SELECTION OF THE DRUG:

For this present study, the metello-mineral formulation **"*KAALAMEGA NARAYANA CHENDHOORAM*"** was taken as the compound drug preparation for oral cancer mentioned in the classical Siddha literature "*Athmarakshamirtham Ennum Vaithiya Saara Sangeraham"* written by *Kandhasamy Mudhaliyaar*, pg no:493,First Edition 1931[94].

## Ingredients of the drug:

1. Purified *Vediuppu [Potassium nitrate ]* – 840 gm
2. Purified *Thurusu [ Copper sulphate ]* – 210 gm
3. Purified *Padikaaram [Aluminium potassium sulphate ( Alum )]* – 840 gm
4. Purified *Vengaram [ Sodium bicarbonate* ( Borax) *]* – 210 gm
5. Purified *Navacharam [Ammonium Chloride ]*-210gm
6. Purified *Pooneeru [Impure Sodium Carbonate (Fullers Earth) ]* – 105 gm
7. Purified *Jaathilingam* [ Red sulphate of mercury ]-525gm
8. Purified *Gandhagam [Sulphur ]* – 420 gm
9. Purified *Kalluppu [Sodium chloride ]*- 210 gm
10. Purified *Rasam [ Hydragyrum ]* – 1050 gm
11. Purified *Aritharam [Tri sulphate of Arsenic* (Yellow Orpiment) *]*- 350 gm
12. Purified *Manosilai [Di suphate of Mercury* (Red Orpiment) *]*- 140gm

## Collection of the raw materials:

All the raw materials were purchased from R.N. Rajan country drug store, Parrys corner, Chennai.

## Identification and Authentication of the drug:

The raw materials were identified and authenticated by the experts of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai- 106.

The specimen sample of each raw material has been kept in the PG *Gunapadam* department individually for future reference.

**Purification of the drugs:**

Purification process was done as per the classical Siddha literature [10j].

**1. Purification of Pottasium Nitrate (*Vediuppu)* :**

**Materials Required:**

1. Salt – 100gm
2. Water – 400gm
3. Fermented butter milk – 100gm
4. Lime juice – 100 gm

**Procedure:**

Water was added to the pottasium nitrate and boiled on a hearth with mild flames. The white yolk of eggs (4 nos ) were added to every 1400gm of salt and the bubbles thus appeared with impure substances were removed with wooden spoon.

The ingredients were then transferred to another pot, sealed with mud pasted cloth, filtered and transferred to another pot, sealed with mud pasted cloth, filtered and kept in places without aeration. Next day the water was filtered and salt was sun shade. This process was repeated for seven times to get it purified.

**2. Purification of *Padikaaram* (Aluminium potassium sulphate ( Alum )**

The alum was dissolved in water and it was filtered, boiled. Then it was cooled to get purified form.

**3. Purification of *Thurusu* (Copper sulphate):**

The copper sulphate was fried, till it turns to whitish.

**4. Purification of *Vengaram (*Sodium biborate*)*:**

Borax was bundled and hanged in the buffalo's dung solution and boiled. The bundle was cleaned with fresh water and insolated to get it in purified form.

**5. Purification of *Navacharam* (Ammonium chloride):**

*Navacharam* (Ammonium chloride) was dissolved in hot water and filtered. After it was cooled, it was poured in a broad mouthed vessel and insolated; the salt

was formed in a purified form. It was preserved with small quantity of the root of jequirity in a bottle.

**6. Purification of *Kalluppu* (*Sodium chloride)*:**

*Kalluppu* was dissolved in vinegar and clean with a cloth, dried in a sunshade.

**7. Purification of *Pooneeru* (*Impure Sodium Carbonate) :***

Fuller's earth 1.3 litre was soaked in dew's water 5.2 litres and allowed to settle. Next morning it was churned well and the outer cream layer was removed. The remaining mixture was in procelin plates and insolated to obtain purified form. This process was repeated for ten times and stored in a bottle.

**8. Purification of *Rasam* (Mercury)**

**Materials Required:**

- Mercury - 35 gm
- Brick powder - 100 gm
- Turmeric powder - 100 gm
- *Acalypha* juice (*Acalypha indica*) - 1.3 litre

**Procedure:**

Mercury was triturated with brick powder and turmeric powder for one hour respectively and washed with water. Then the Mercury was boiled with the juice of Indian *Acalypha* till the juice completely evaporates. And thus mercury was purified.

**9. Purification of *Lingam* (Cinnabar):**

Lime juice, cow's milk and the *Acalypha indica* juice were mixed together in equal proportion and allowed to fuse Cinnabar so as to get it in a purified potent form.

**10. Purification of *Thaalagam* ( Yelow Orpiment):**

**Materials required:**

- Arsenic trisulphide – 35 gm
- Cow's urine – 1 litre
- Indian *acalypha* juice – 300 ml

- Lime stone – 300 gm

**Procedure:**

Arsenic trisulphide was bundled and kept immersed in the mixture of limestone, *Acalypha indica* juice and cow's urine and heated to get purified.

**11. Purification of *Gandhagam* (sulfur):**

**Materials Required:**

Sulphur – 35 gm

Butter – 35 gm

Cow's milk – 150 ml

**Procedure:**

Sulphur was placed in an iron spoon. Butter was added and the spoon was heated till the butter melts, this mixture was immersed in inclined position in cow's milk. The procedure was repeated for about 7 times and thus sulphur was purified. Fresh milk was used each time.

**12. Purification of *Manosilai* (Red orpiment)**

**Materials required:**

Red orpiment – 35 gm

Cow's butter milk – 125 ml

**Procedure:**

Red orpiment was triturated with cow's butter milk for 3 hours. It was dried to get purified form[94a].

## 4.1. Preparation of the trial drug – "*KAALAMEGA NARAYANA CHENDHOORAM*"

1. Purified *Vediuppu [Potassium nitrate ]* – 840 gm
2. Purified *Thurusu [ Copper sulphate ]* – 210 gm
3. Purified *Padigaram* [Aluminium potassium sulphate ( Alum )] – 840 gm
4. Purified *Vengaram [ Sodium bicarbonate* ( Borax) *]* – 210 gm
5. Purified *Navacharam [Ammonium Chloride ]*-210gm
6. Purified *Pooneeru [Impure Sodium Carbonate (Fullers Earth) ]* – 105 gm
7. Purified *Jaathilingam* [ Red sulphate of mercury ]-525gm
8. Purified *Gandhagam [Sulphur ]* – 420 gm
9. Purified *Kalluppu [Sodium chloride ]*- 210 gm
10. Purified *Rasam [ Hydragyrum( Mercury) ]* – 1050 gm
11. Purified *Aritharam [Tri sulphate of Arsenic* (Yellow Orpiment) *]*- 350 gm
12. Purified *Manosilai [Di suphate of Mercury* (Red Orpiment) *]*- 140gm

**Procedure:**

- 840 gm of 8$^{th}$ solution of *Vediuppu [Potassium nitrate ]* and *Padigaram* [Aluminium potassium sulphate *(Alum) ]* were taken
- Along with that, 210 gm of *Thurusu [ Copper sulphate ]*, *Vengaram [ Sodium bicarbonate* (Borax)*]*, *Navacharam [Ammonium Chloride ]*, *Kalluppu [Sodium chloride Impura ]* were taken and then mixed with 105 gm of *Pooneeru* [*[Impure Sodium Carbonate (Fullers Earth) ]*.
- Above ingredients were ground into fine powder and divided into 3 parts
- First part of the powder was underwent distillation process, the end product was mixed with 2$^{nd}$ part of powder and dried.
- Second part of the powder was underwent distillation process, the end product was mixed with 3$^{rd}$ part of powder and dried.
- Third part of the powder was undergoes distillation process, the final end product was taken and kept in a sealed bottle.

- The *Jaathilingam* [ Red sulphate of mercury ]-525 gm, *Aritharam* [Tri sulphate of Arsenic (Yellow orpiment)]-350 gm, *Gandhagam [Sulphur ]* 420 gm , *Manosilai [* Di sulphate of mercury (Red Orpiment) *]* 140 gm were ground ,along with the end product of distillation for 12 hours (4 *saamam*) and made into fine powder and dried.
- Dried powder was kept in a mud pot which was sealed with 7 mud pasted plaster.
- Another mud pot with small quantity of sand was taken and above preparation was kept into it and sealed the lid with mud pasted plaster.
- The mud pot was ignited by using *Aavarai* stick for 30 hours ( 10 *saamam*), after 30 hours "*Chendhooram*" was obtained.

**Drug profile:**

- Drug name : *Kaalamega Narayana Chendhooram*
- Dosage : 244 mg of *Chendhooram* [1/2 *Panavedai* ]
- Route : Enteral (oral)
- Adjuvant : *Thipili chooranam* with honey (bd for 48 days – 1 *mandalam*)
- Indications : *Kannaputru* [ ORAL CANCER ], *Elaippu* [Tuberculosis], *Kuttam 18* [ Hansen´s Disease]
- Reference : *"AthmarakshaMirutham Ennum Vaithiya Saara Sangeeraham*".

**Fig no: 13. Ingredients of *Kaalamega Narayana Chendhooram* :**

**Purified *Vediuppu [Potassium nitrate ]***

**Purified *Thurusu [ Copper sulphate ]***

**Purified *Padigaram* [Aluminium potassium sulphate ( Alum )]**

**Purified *Vengaram [ Sodium bicarbonate(Borax)]***

**Purified *Navacharam [Ammonium Chloride ]***

**Purified *Kalluppu [Sodium chloride Impura ]***


**Purified *Pooneeru [Impure Sodium Carbonate (Fullers Earth)]***


**Purified *Rasam [ Hydragyrum ]***


**Purified *Jaathilingam* [ Red sulphate of mercury]**


**Purified *Aritharam* [Tri sulphate of Arsenic (Yellow orpiment)]**


**Purified *Gandhagam [Sulphur]***


**Purified *Manosilai [ Red Orphiment ]***

**Fig no: 14. Preparation of *Kaalamega Narayana Chendhooram*:**

**Process 1:**

**Preparing for *Thravagam***

**Process 2.**

**Divided into 3 parts**

**Process 3.**

**1st part undergoes distillation process**

**Collection of *Thravagam***

**Process 4.**

**The obtained *Thravagam* is used underwent distillation to grind the second part**

**Again the second part process**

**Process 5.**

**The obtained *Thravagam* is used distillation to grind the third part**

**Again the third part underwent process**

**Process 6.**


**The end product of distillation of distillation was sealed in a bottle.**

**Process 7.**

**Grinding of prepared medicine**

**Process 7.**

**Final product was sealed with mud pasted cloth**

**Process 8.**

**Ignition of final *Chendhooram***

**Final end product of *Chendhooram***

***Chendhooram***

**Analysis as per AYUSH guidelines[95]**

**1. Floating on Water:**

A pinch of *Chendhooram* gently placed on the still surface of water in a vessel, did not sink immediately. It was found that the *Kaalamega Narayana Chendhooram* particles floated over the surface of water indicated lightness of the trial drug.

**2. Lines on fingers:**

*Chendhooram* in well prepared form should be as fine powder. When taken between thumb and index finger, the fine powder will fill up the lines of the finger print. A pinch of *Kaalamega Narayana Chendhooram* was taken in between the thumb and index finger and rubbed. It was found that the *Kaalamega Narayana Chendhooram* entered into the lines of the finger and was not easily washed out from the lines, confirmed its fineness.

**3. Irreversible reaction**:

The well prepared *Chendhooram* does not get reversible to its metallic state when heated with a mixture of cane jaggery, hemp powder, ghee and honey. A pinch of *Kaalamega Narayana Chendhooram* was taken and mixed with cane jaggery, ghee and honey. It was observed that *Kaalamega Narayana Chendhooram* did not reverse to its metallic state.

**4. Tasteless:**

The well prepared *Chendhooram* should be completely tasteless. Presence of any taste like sweet or bitter indicate incomplete preparation which needed another Calcination process. When a small amount of *Kaalamega Narayana Chendhooram* was kept on the tip of the tongue, no specific taste was found.

**5. Lusterless:**

If any shining particle is present in *Chendhooram*, it indicates that the *Chendhooram* is not manufactured properly and contains unchanged substances like minerals, metals and other toxic substances. There should be no shining particles present in the well manufactured *Chendhooram*. *Kaalamega Narayana Chendhooram* was taken in a petri bowl and observed for any lustre in daylight through magnifying glass. No lustre was observed in the *Chendhooram*.

## DRUG STANDARDISATION:

Standardisation of drug means confirmation of its identity, determination of its quality, purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological evaluations [95].

## STANDARDIZATION OF THE DRUG *KMNC:*

Standardization of drugs helps to prove its identity and determination of its quality and potency. Standardization of the Metallo-mineral formulation is based on the qualitative and quantitative analysis through Physico-chemical investigations and instrumental analysis. The Physico-chemical analysis of the prepared Metalo--mineral drug have been done at Central Research Institute, Arumbakkam, Chennai and elemental analysis have been done at IIT, Chennai. (FTIR, SEM, ICP-MS, XRD)[96]

### Method of standardization:

Techniques Involved In Standardization of Compound Drugs:

- Macroscopic Methods
- Microscopic Methods
- Physical Methods
- Chemical Methods
- Biological Methods

### Organoleptic character of the *Chendhooram* :

- The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odour, taste, texture etc.

**Colour:**

- A sample of *Chendhooram* were taken in watch glasses and placed against white back ground in white tube light. The *Chendhooram* were observed for its color by naked eye.

**Odour:**

- *Chendhooram* were smelled, the time intermission between two smelling was kept 2 minutes to nullify the effect of previous smelling.

**Taste**:

- A sample of about *Chendhooram* was tasted and the taste was reported.

**Size:**

- The *chendhooram* was completely sieved through mesh size 120.

### 4.2.1. Physico-Chemical Investigations[96]:

Physico-chemical investigations like pH value, Loss on drying at 105°C, Ash test have been done at The Tamilnadu Dr M.G.R Medical University, Anna salai, Guindy, as per the guide lines of WHO.

**Solubility Test:**

A pinch of sample (*KMNC*) was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Petroleum ether, Propylene glycol, Toluene, Benzene, Chloroform, Ethyl alcohol, Xylene, Carbon tetra chloride and the results are observed individually.

**pH value:**

Potentio metrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the *KMNC* was written in results column.

**Loss on Drying:**

An accurately weighed 2gm of *Kaalamega Narayana Chendhooram* formulation was taken in a tarred glass bottle. The crude drug was heated $105^0$ c for 6 hours in an oven till a constant weight. The percentage moisture content of the sample was calculated with reference to the shade dried material.

**Determination of total Ash:**

Weighed accurately 2g of *Kaalamega Narayana Chendhooram* formulation was added in crucible at a temperature $600^0$c in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

**Determination of acid insoluble ash:**

Ash above obtained was boiled 5min with 25ml of 1M hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

**Determination of water soluble ash:**

Total Ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with water and ignited for 15 min at a temperature not exceeding $450^0$c in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

**Determination of water soluble extractive**:

5gm of air dried drug. Coarsely powered *Kaalamega Narayana Chendhooram* was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 1000c and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

**Determination of alcohol soluble extractive:**

2.5gm of air dried drugs coarsely powdered *Kaalamega Narayana Chendhooram* was macerated with 50ml. alcohol in closed flask for 24 hours. With frequent shaking, it was filtered rapidly talking precaution against loss of alcohol .10ml of filtrate was the evaporated in a tarred flat bottom shallow dish, dried at 1000 c and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

**BIO-CHEMICAL ANALYSIS[97]**

The bio-chemical analysis was done to identify the acid and basic radicals present in the *KMNC*.

**Preparation of extract**

5g of *KMNC* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

### 4.2.2. PRELIMINARY BASIC AND ACIDIC RADICALS

**Test for basic radicals**

**1. Test for Potassium**

To a pinch of the *KMNC* 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

**2. Test for Calcium**

To 2 ml of *KMNC* extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate**.**

**3. Test for Magnesium:**

To 2ml of *KMNC* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

**4. Test for Ammonium:**

To 2ml of *KMNC* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

**5. Test for Sodium**

Hydrochloric acid was added with a pinch of the *KMNC*, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. **Test for Iron (Ferrous)**

The *KMNC* extract was treated with Conc. $HNO_3$ and ammonium thiocyanate and waited for the appearance of blood red colour**.**

**7. Test for Zinc**

To 2 ml of the *KMNC* extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

**8. Test for Aluminium**

To the 2m1 of the *KMNC* extract sodium hydroxide was added in drops and changes are noted.

**9. Test for Lead**

To 2 ml of *KMNC* extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate**.**

**10. Test for Copper**

a. A pinch of *KMNC* was made into a paste with conc. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of *KMNC* extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

**11. Test for Mercury**

To 2m1 of the *KMNC* extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

**12. Test for Arsenic**

To 2 ml of the *KMNC* extract 2ml of sodium hydroxide solution was added and brown or red precipitate formation was noted.

**Test for acid radicals**

**1. Test for Sulphate**

To 2 ml of the *KMNC* extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

**2. Test for Chloride**

The *KMNC* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

**3**. **Test for Phosphate**

The *KMNC* extract was treated with ammonium molybdate and conc. $HNO_3$ and observed for the appearance of yellow precipitate.

4. **Test for Carbonate**

The *KMNC* extract was treated with conc.HCl and observed forth appearance of effervescence.

**5. Test for Fluoride & Oxalate:**

To 2ml of *KMNC* extract 2ml of dilute acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

**6. Test for Nitrate:**

To 1 gm of the *KMNC*, copper turnings was added and again conc.$H_2SO_4$ was added, heated and the test tube was tilted vertically down and observed for any changes.

**4.2.3. MICROBIAL LOAD[98]**

**AVAILABILITY OF MICROBIAL LOAD:**

**Enumeration of bacteria by plate count – agar plating technique**

The plate count technique was one of the most routinely used procedures because of the enumeration of viable cells by this method.

**Principle:**

This method is based on the principle that when material containing bacteria are cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies therefore is the same as the number of organisms contained in the sample.

**Dilution:**

A small measured volume is mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilution is usually made in multiples of ten. A single dilution was calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluent}}$$

**Requirements:**

- Sample or Bacterial suspension
- 9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes (7)
- Nutrient agar medium (200 ml)
- Colony counter.

**Procedure:**

1. Label the dilution blanks as $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$.
2. Prepare the initial dilution by adding 1 ml of the sample into a 9 ml dilution blank labeled $10^{-1}$ thus diluting the original sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank $10^{-2}$ with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the $10^{-2}$ suspension, transfer 1 ml of suspension to $10^{-3}$ dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the original sample has been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes.
8. Three petri dishes are to used for each dilution.
9. Add approximately 15 ml of the nutrient medium, melted and cooled to $45^0$c, to each petri dish containing the diluted sample.

   Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
10. Allow the plates to solidity.
11. Incubate these plates in an inverted position for 24-48 hours at $37^0$c.

**Observation:**

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 replates)}}{\text{Amount of plated} \times \text{dilution}}$$

### 4.2.4. SOPHISTICATED INSTRUMENTAL ANALYSIS

**FT IR - Fourier Transform Infra-red Spectroscopy[99]**

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterise some inorganics. Examples include paints, adhesives, resins, polymers, coatings and **drugs**. FTIR is an effective analytical instrument for detecting functional groups.

**Fig no:15 FTIR INSTRUMENT**

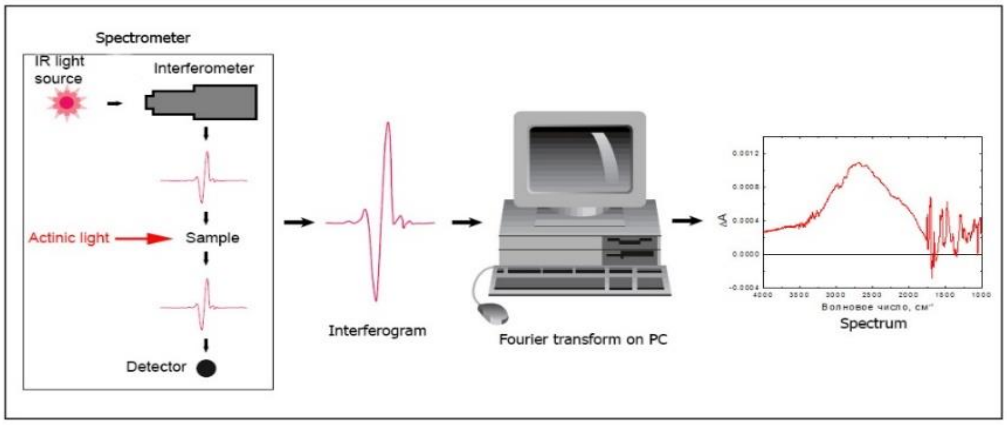



**Fig no:16 FTIR MECHANISM**

**APPLICATIONS**:

Quantative scans

Qualitative scan solids, liquids, gasess

Organic samples, inorganic samples

Unknown identification

Impurities screening

Formulation

Pharmaceuticals

**Principle:**

Spectrophotometric tests are commonly used in the identification of chemical substances and quantification of polymorphic forms. The test procedures are applicable to substances that absorb IR radiation. The IR absorption spectrum of a substance compared with that obtained concomitantly for the corresponding reference standard / reference substance provide conclusive evidence of the identity of the substance being tested.

**Recording Infrared spectrum of a solid as a disc (as per USP ‹197K›):**

- Triturate about 1 to 2 mg of the substance to be examined with 300 to 400 mg, unless otherwise specified, of finely powdered and dried potassium bromide. If the substance is a hydrochloride it is preferable to use potassium chloride.
- Carefully grind the mixture and spread it uniformly in a suitable die.
- Submit it to the pressure of about 800 mPa (8 tons/cm2).
- Examine the disc visually and if any lack of uniform transparency is observed, reject the disc and prepare again.
- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.
- When sample and standard are measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10% between them ( For eg. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

**SEM (SCANNING ELECTRON MICROSCOPE)[100]**


**Fig no:17 SEM INSTRUMENT**

## DEFINITION

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects [83].

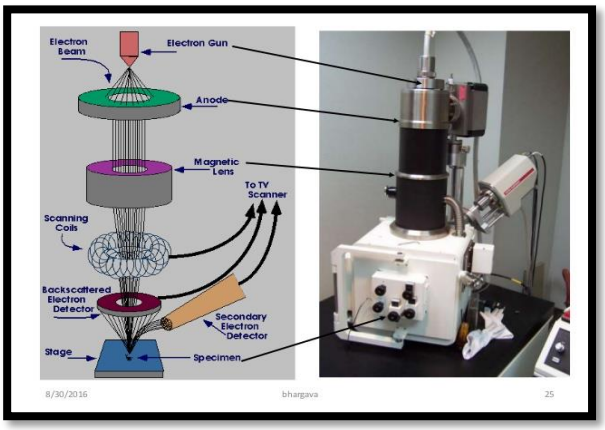



**Fig no:18 SEM MECHANISM**

## SEM ANALYSIS APPLICATIONS

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

**External morphology (texture)**

Chemical composition (when used with EDS)

Orientation of materials making up the sample

**The EDS component of the system is applied in conjunction with SEM analysis to:**

Determine elements in or on the surface of the sample for qualitative information.Measure elemental composition for semi-quantitative results.Identify foreign substances that are not organic in nature and coatings on metal.

SEM Analysis with EDS – qualitative and semi-quantitative results Magnification – from 5x to 300,000x.Sample Size – up to 200 mm (7.87 in.) in diameter

and 80 mm (3.14 in.) in height.Materials analysed – solid inorganic materials including metals and minerals.

**THE SEM ANALYSIS PROCESS**

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample. Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers. In scanning electron microscope high-energy electron beam was focused through a probe towards PP. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector [84].

The types of signal produced by a scanning electron microscope include

- Secondary electrons
- back scattered electrons
- characteristic x-rays light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

**ICP-MS - Inductively Coupled Plasma Mass Spectrometry[101]**

**Analysis of Trace Metal and Inorganic Materials**

Inductively Coupled Plasma Mass Spectrometry is a technique routinely used to analyze trace levels of a wide range of inorganic elements. The ICP-MS allows for the detection and quantification of elements with atomic mass ranges 7 to 250. This covers Lithium to Uranium.

The typical detection limits are in the parts per billion (ppb) range and even parts per trillion (ppt) in some cases. The ICP-MS analysis methods available at LPD Lab Services allow the detection, identification and quantification of a wide array of elements using a Perkin Elmer ELAN 6000 ICP-MS

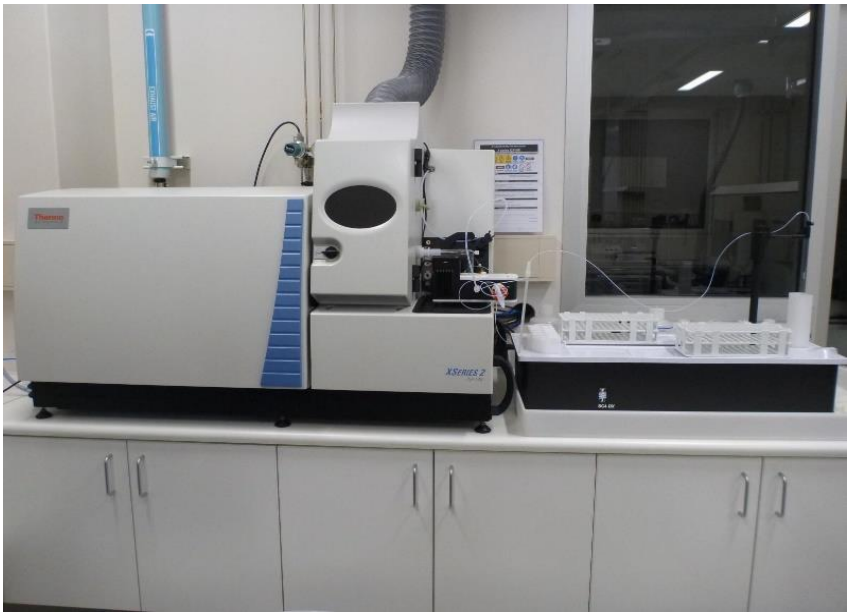


**Fig no:19 ICPMS- INSTRUMENT**

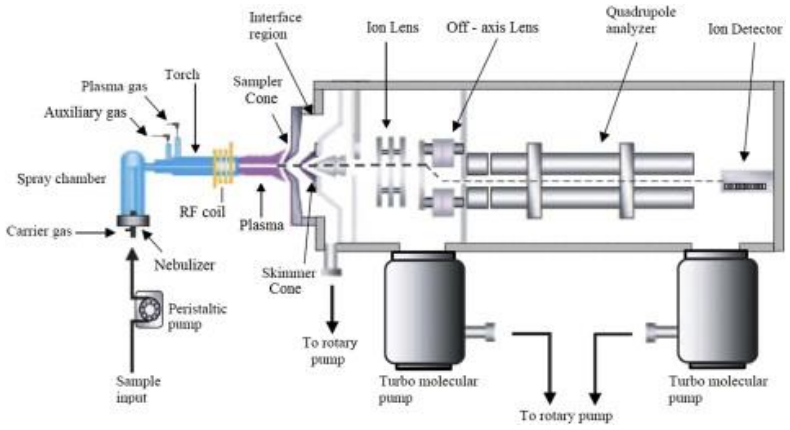



**Fig no:20 ICP MS MECHANISM**

Analysis: Analyze according to the manufacturer's suggestions for program and m/z. Calculate and report results based on the original sample size.

**Applications of ICP-MS**

- Monitoring of trace metals in drinking water, ground water, rainwater, wastewater or industrial effluent streams.
- Trace elements in product / raw materials or from washed or rinsed surfaces.
- Analysis of additives and purity in metal alloys.
- Analysis of low level contaminants in chemical products, beverages, foods, cosmetics, pharmaceuticals.
- Analysis of soluble / leachable material from solid samples such as medical devices, polymers, PCB`s.
- Analysis can be performed on a diverse range of sample

**XRD - X-ray Powder Diffraction (XRD)[102]**

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined.

**DEFINITION**

X-ray powder diffraction is most widely used for the identification of unknown

crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

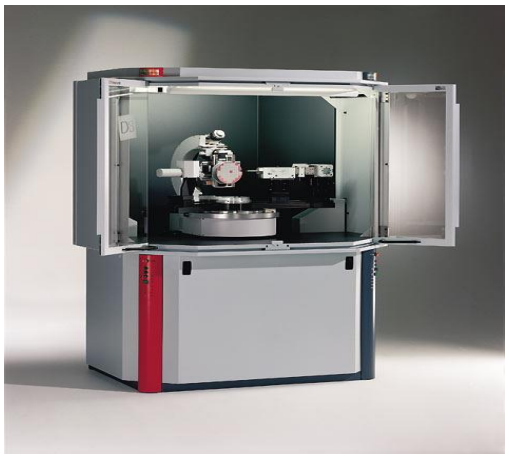


**Fig no:21 XRD - X-ray Powder Diffraction**

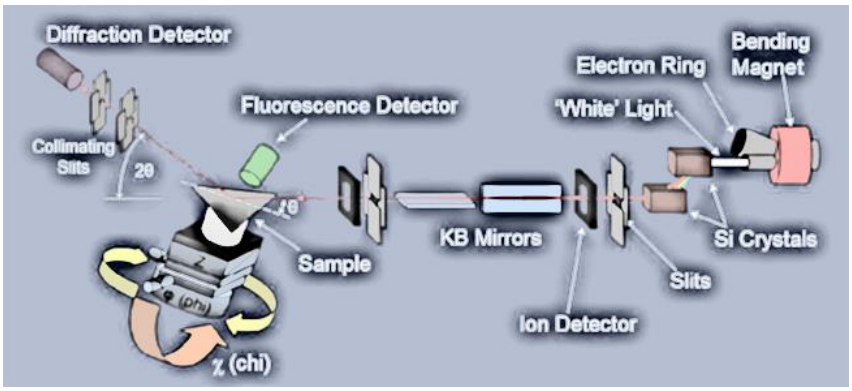



**Fig no:22 XRD Mechanism**

**APPLICATIONS:**

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

**With specialized techniques, XRD can be used to:**

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)

**Characterize thin films samples by:**

- Determining lattice mismatch between film and substrate and to inferring stress and strain
- Determining dislocation density and quality of the film by rocking curve measurements
- Measuring super lattices in multilayered epitaxial structures
- Determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

**Strengths and Limitations of X-ray Powder Diffraction:**

**Strengths:**

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

**Limitations:**

- Homogeneous and single phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

**Sample Collection and Preparation**:

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible

- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation. Powder less than ~10 μm(or 200-mesh) in size is preferred
- Place into a sample holder or onto the sample surface.

### 4.2.5. TOXICOLOGICAL STUDIES[103]

## ACUTE ORAL TOXICITY STUDY OF *KAALAMEGA NARAYANA CHENDHOORAM*

**(OECD GUIDELINE – 423)**

**Introduction:**

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.
- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.
- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

**Principle of the Test:**

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute

toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

− no further testing is needed

− dosing of three additional animals, with the same dose

− dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

## Methodology:

**Selection of Animal Species**

The preferred rodent species is the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

**Housing and Feeding Conditions**

The temperature in the experimental animal room should be 22ºC ± 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

## Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

**Test Animals and Test Conditions:**

Sexually mature Female Wistar albino rats (150-200gm) were obtained

from TANUVAS, Madhavaram, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

## Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, ***Kaalamega Narayana Chendhooram (Kmnc)***

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

### IAEC approved Number: IAEC/XL VIII/13CLBMCP/2016

| | |
|---|---|
| **Test Substance** | :*Kaalamega Narayana Chendhooram (Kmnc)* |
| **Animal Source** | : TANUVAS,Madhavaram, Chennai. |
| **Animals** | : Wister Albino Rats (Female-3+3) |
| **Age** | **:** 6-8 weeks |
| **Body Weight on Day 0** | **:** 150-200gm. |
| **Acclimatization** | **:** Seven days prior to dosing. |
| **Veterinary examination** | **:** Prior and at the end of the acclimatization period. |
| **Identification of animals** | **:** By cage number, animal number and individual marking by using Picric acid. |
| **Number of animals** | : 3 Female/group, |
| **Route of administration** | **:** Oral |
| **Diet** | **:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore |
| **Water** | **:** Aqua guard portable water in polypropylene bottles. |
| **Housing & Environment** | **:** The animals were housed in Polypropylene cages provided with bedding of husk. |
| **Housing temperature** | **:** between 22ºC ± 3ºC. |
| **Relative humidity** | **:** between 30% and 70%, |
| **Air changes** | **:** 10 to 15 per hour and |
| **Dark and light cycle** | **:** 12**:**12 hours. |
| **Duration of the study** | **:** 14 Days |

**Administration of Doses:**

*Kaalamega Narayana Chendhooram (KMNC)* was suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 5, 50, 300 and 2000 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitered for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

**Limit test**

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

**Observations:**

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

**a. Mortality**

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

**b. Body weight**

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

**C. Cage-side observation**

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioral patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

**d. Gross necropsy**

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

**Histopathology**

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

**Data and reporting**

All data were summarized in tabular form, (Table-1-4) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight changes, food and water intake.

**Test substance and Vehicle**

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *Kaalamega Narayana Chendhooram (KMNC)* with 2% CMC solution and it was found suitable for dose accuracy.

**Justification for choice of vehicle**

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique [104].

## REPEATED DOSE 28-DAY ORAL TOXICITY (407) STUDY OF *KAALAMEGA NARAYANA CHENDHOORAM (KMNC)*[105]

| | |
|---|---|
| **Test Substance** | : *Kaalamega Narayana Chendhooram (KMNC)* |
| **Animal Source** | : TANUVAS, Madhavaram, Chennai. |
| **Animals** | : Wister Albino Rats (Male -24, and Female-24) |
| **Age** | **:** 6-8 weeks |
| **Body Weight** | **:** 150-200gm. |
| **Acclimatization** | **:** Seven days prior to dose. |
| **Veterinary examination** | **:** Prior and at the end of the acclimatization period. |
| **Identification of animals** | **:** By cage number, animal number and individual marking by using Picric acid |
| **Diet** | **:** Pellet feed supplied by Sai Meera Foods Pvt Ltd, Bangalore. |
| **Water** | **:** Aqua guard portable water in polypropylene bottles. |
| **Housing & Environment** | **:** The animals were housed in Polypropylene cages provided with bedding of husk. |
| **Housing temperature** | **:** between 22ºC±3ºC. |
| **Relative humidity** | **:** between 30% and 70%, |
| **Air changes** | **:** 10 to 15 per hour |
| **Dark and light cycle** | **:** 12**:**12 hours. |
| **Duration of the study** | **:** 28 Days. |

**Justification for Dose Selection:**

The results of acute toxicity studies in Wistar albino rats indicated that *Kaalamega Narayana Chendhooram (KMNC)* was non-toxic and no behavioural changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected As per OECD guideline three dose levels were selected for the study. They are low dose (5X), high dose (10X). X is calculated by multiplying the acute toxicity dose (2000mg) and the body surface area of the rat (0.018), 5X dose is (10mg/kg), 10X dose is (20mg/kg) The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

**Preparation and Administration of Dose:**

*Kaalamega Narayana Chendhooram (KMNC)* suspended in with water, It was administered to animals at the dose levels of 5X, 10X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

**Methodology**

| **Groups** | **No of Rats** |
|---|---|
| 1. Group I Vehicle control | 20(10male, 10female) |
| 2. Group II KMNC - low dose X (20mg) | 20(10male, 10female) |
| 3. Group III KMNC - Mid dose 5X (100mg) | 20(10male, 10female) |
| 4. Group IV KMNC – High dose 10X (200mg ) | 20(10male, 10female) |

**Randomization, Numbering and Grouping of Animals:**

80 Wistar Albino Rats (40M + 40F) were selected and divided into 4 groups. Each group consist of 20 animals (Male -10 and Female-10). First group treated as a control and other three group were treated with test drug (low, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

**Observations:**

**Experimental animals were kept under observation throughout the course of study for the following:**

**Body Weight:**

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study. From the data, group mean body weights and percent body weight gain were calculated.

**Food and water Consumption:**

Food and water consumed per animal was calculated for control and the treated dose groups.

**Clinical signs**:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

**Mortality:**

All animals were observed twice daily for mortality during entire course of study.

**Functional Observations**:

At the end of the 4$^{th}$ week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

**Laboratory Investigations:**

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Biochemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

**Hematological Investigations**

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

**Biochemical Investigations**

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods.

Activities of glutamate oxaloacetate transaminase/Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

**Necropsy:**

All the animals were sacrificed by excessive anesthesia on day 29. Necropsy of all animals was carried out.

**Histopathology:**

Histopathological investigation of the vital organs was done. The organ pieces (5-6µm thick) of the highest dose level of 300 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Hematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to Histopathological examination.

**Statistical analysis:**

Findings such as body weight changes, water and food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed

by dunnet'S multi comparison test using a computer software programme – GRAPH PAD VERSION-7 version.

## 4.3. PHARMACOLOGICAL ACTIVITY:

### 4.3.1. ANTICANCER ACTIVITY:

**INVITRO ANTICANCER EFFECT DETERMINATION BY MTT ASSAY[106]**

KB (Oral Carcinoma) cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's modified Eagles medium, DMEM ( Sigma aldrich, USA).

The cell line was cultured in 25 $cm^2$ tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% $CO_2$ incubator (NBS Eppendorf, Germany).

The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

**Cells seeding in 96 well plate:**

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% $CO_2$ incubator.

**Preparation of compound stock:**

1mg of sample was weighed and dissolved in 1mL DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

**Anticancer Evaluation:**

After 24 hours the growth medium was removed, freshly prepared each compounds in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of 5% DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% $CO_2$ incubator. Non treated control cells were also maintained.

**Anticancer Assay by Direct Microscopic observation:**

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Anticancer Assay by MTT Method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization..

After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% $CO_2$ incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide,DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

**APPENDIX**

**Instruments and reagents used:**

| | |
|---|---|
| DMEM media | -Sigma Aldrich, USA D5648 |
| Fetal Bovine Serum | -Gibco, US orgin- |
| 0.25% Trypsin | - Invitrogen, USA 25200-056 |
| Micropipettes | - F1 Thermoscientific USA |
| $CO_2$ Incubator | - Eppendorf, GERMANY |
| Phase Contrast Microscope | - Olympus, JAPAN with Optika Pro 5 Camera |
| MTT | - Sigma Aldrich M5655 |

ELISA Reader - ERBA, GERMANY

Culture Plates and Flasks - NUNC, Thermoscientific USA

Image Magnification - 10X


**Fig no:23 Microplate Reader**

**Fig no:24 $CO_2$ Incubator**

### 4.3.2. IN VITRO ANTI- TUMOUR ACTIVITY

### CELL CULTURE MATERIAL

OSCC cell lines were purchased from American Tissue Collection Centre (ATCC) and were maintained in Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F12 (DMEM/F12) (Sigma-Aldrich, USA).HOS cell lines were purchased from ATCC and were maintained in DMEM high glucose. Culture media was supplemented with 10% Foetal Bovine Serum (FBS) and 1% penicillin/streptomycin.

**Cell apoptosis assay by flow cytometry**

Cellular apoptosis was determined using the AnnexinV-FITC Apoptosis Detection Kit I (Clontech Laboratories Inc, USA) according to the manufacturer's protocol. OSCC and HOS cell lines were cultured at 6 × 10cells/ml and seeded in 60 mm dish. The cells were treated with free medium containing various concentrations of ABC for 6, 12 and 24 hour. Cells were harvested by trypsinization, then washed twice with cold PBS and centrifuged at 1000 rpm. About $1 \times 10^5$ -$1 \times 10^6$cells were then re suspended in 400 µl1×binding buffer, centrifuged again at 1000 rpm for 5 minutes and then supernatant was removed. Cells were re-suspended in 200 µl 1× binding buffer and transferred to a sterile flow cytometry glass tube. Five µl Annexin V-FITC and 10 µl propidium iodide were added and then incubated in the dark at room temperature. Cells were analyzed by flow cytometer at 488 nm. The distribution of cells was analyzed using Cell Quest software (Becton-Dickinson) in the flow cytometer within 1 hour of staining. Data from 10,000 cells was collected for each data file. Apoptotic cells were identified as Annexin V-FITC-positive and P-negative cells

### 4.3.3.ANTIMICROBIAL ACTIVITY[107]:

### AGAR- WELL DIFFUSION METHOD:

### PRINCIPLE:

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

## MATERIALS REQUIRED:

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HI Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFards Standard, 0.5%

1. *E.coli* (ATCC 25922)
2. *Staphylococcus aureus* (ATCC 25923)
3. *Pseudomonas aeroginosa* (ATCC 27853)
4. *Streptococcus mutans* (MTCC 890)
5. *Klebsiella pneumonia* (ATCC 13883)

## PROCEDURE

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E.coli, Streptococcus mutans, Pseudomons aeroginosa, Klebsiella pneumoniae a*nd *Staphylococcus aureus* (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg/mL, 500µg/mL, 1000µg/mL were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

## 5. RESULTS AND DISCUSSION

One of the *Siddha* metalo-mineral formulation, *Kaalamega Narayana Chendhooram* had been exposed to several modern scientific studies to establish its efficacy to scientific people and public. Literary collection, Physicochemical and elemental analysis, toxicological studies and pharmacological studies were done to justify the anticancer activity of *KMNC* against Oral cancer. *Siddha* literatures related to the drug brings the evidence and importance of its utility in treating the cancer. The desirable consequences are displayed and discussed for its anticancer nature.

- *Gunapadam* review brings the effectiveness of the drug in treating cancer.
- *Siddha* and Modern aspects of the disease were also reviewed. Disease review brings about the knowledge of the disease.
- The pharmacological review explains about the methodology of Anti-cancer activity and the drugs used.
- Pharmaceutical review describes about the *Chendhooram*, its properties and its efficacy in treating cancer.
- Lateral research gives the effectiveness of the drug in treating cancer.

**Discussion on *Gunapadam* review**

- The poem for general properties of processed quicksilver directly indicates its anticancer nature.
- *Padigaram* had a property of healing chronic ulcers.
- *Vengaram* was used in the treatment of ulcers.
- *Thurusu* was used for its healing deep chronic ulcers.
- *Navachaaram* is an ingredient o*f veera mezhugu* shows a potent anticancer effects.
- *Lingam* had a property of healing deep ulcer.

**Discussion on modern drug review**

- Alum had an anti - microbial activity, in which microbes also plays an important role in causing cancer[85a]
- Mercury helps to destroy the cancer cells and reduces the tumour growth [89a].
- Copper sulphate, Cinnabar, Orpiment have antimicrobial activity[86a]

- Borax have anti-inflammatory and healing properties[87a]
- Orpiment plays an important role in treating blood cancers[91a]
- Sulphur have an anticancer effect[92a]
- Arsenic exhibits anti -cancer activity [93a].

**Discussion of pharmacological review**

The cell lines for this anticancer activity evaluation was carried out using KB cell line (oral cancer cell lines). They are the genomes of HPV 16 and HPV 18 respectively. These HPV 16 and HPV 18 are the responsible for 93% of Oral Cancer [3a].

So, the analysis of pharmacological activity through KB cell lines is the novel methods for validation. They explained effective anticancer character of *KMNC.*

**Discussion on pharmaceutical review**

- *Chendooram*.
- 75 years of shelf life denotes its long time efficacy.
- Being very fine particles it increases the therapeutic effect.

**Discussion on materials and methods**

The selection of trial drug was taken from the book *Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham* written by *Kandhasamy Mudhaliyaar*, was approved by the Department of AYUSH as Per the Classical Siddha literature.

The ingredients were bought from the authenticated vender and they were identified and authenticated by the experts in Post Graduate Department *Gunapadam*, GSMC, Chennai. So the ingredients were perfect and original.

The preparation of medicine was done at the well-equipped lab of the Post Graduate Department of *Gunapadam*.

The analytical parameters were conducted at registered and licensed laboratories only. Thus the result of *Kaalamega Narayana Chendhooram* under various analytical procedures showed the accuracy of it.

The *Siddha* metalo-mineral formulation *Kaalamega Narayana Chendhooram* had been subjected to various studies for its scientific validation and safety assessment.Literary collections, physicochemical and Elemental analysis, Toxicological study, Pharmacological studies are done to prove its efficacy.

## Results of Siddha Standardization

**Table:7**

| **S.No** | **Parameters** | **Results for ideal *Chendhooram*** | **Results of *KMNC*** | **Interpretation** |
|---|---|---|---|---|
| 1 | Colour | Reddish | Reddish brown | *Chendooram* colour. |
| 2 | Floating of water | Floats on water | Floats on water | Lightness of drug. |
| 3 | Finger Print test | Impinged in the furrows of finger | Impinged in the furrows of finger | Indicates fine particles of powder. |
| 4 | Luster | Lusterless | Lusterless | Change of specific metallic character of raw material after incineration |
| 5 | Taste | No specific taste | No specific taste | Change of specific metallic character of raw material after incineration |

**Colour:**

It is reddish brown in colour. The absence of shining indicates there is no free form of metals.

**Fig no: 25**

**Floating on water:**

*Kaalamega Narayana Chendhooram* floats on water. It is due to its less specific gravity. So, it possesses the property of *Chendhooram.*

**Fig no: 26**

**Finger print test:**

*Kaalamega Narayana Chendhooram* impinged on the cervices of finger. This indicates the particles are fine and it is in micro size.

**Fig no: 27.**

**Lusterless& tasteless:**

It is lusterless and tasteless

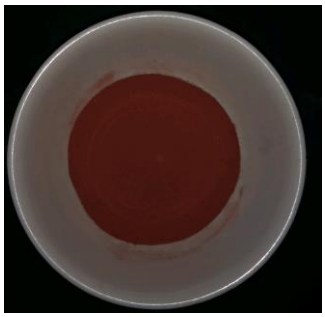


**Fig no: 28.**

**Physical characterization of *Kaalamega Narayana Chendhooram***

**Table-8**

| S.no. | Parameter | Result |
|---|---|---|
| 1. | Colour | Reddish brown in colour |
| 2. | State of the drug | Powder |
| 3. | Consistency | Fine powder |
| 4. | Solubility | Sparingly soluble in water, DMSO.<br>Well soluble in acids (Hcl and $H_2So_4$) |
| 5. | Sense on touch | Fine |
| 6. | Sense on taste | Tasteless |
| 7. | Sense of smell | No significant smell is observed |

## Results of Physical Parameters

**Table-9**

| S.NO | Parameter | Result |
|---|---|---|
| 1. | Specific gravity | 0.956 |
| 2. | pH value | 4.24 |
| 3. | Particle size | Completely passes through sieve no.120 |
| 4. | Loss on drying at 105 degree Celsius | 0.61% |
| 5. | Total ash value | 97.78% |
| 6. | Acid insoluble ash | 0.23% |
| 7. | Water soluble ash | 4.76% |

**Discussion on Physico - Chemical parameters:**

**Solubility**

- Solubility is the major factor for the bioavailability of a drug substance.
- It is useful to determine the form of drug and processing of its dosage form.
- The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability [108].

*KMNC* is soluble in major solvents ($H_2SO_4$, HCl) and sparingly soluble in water proves that its efficiency of solubility in the stomach indirectly, increasing the bio availability.

**Specific gravity**

The trial drug "*Kaalamega Narayana Chendhooram"* shows (0.956) low specific gravity compared to water. Thus it flows in water and indicates lightness of the medicine. This lightness of the medicine indicates its nature of absorption.

**pHvalue**

- *Kaalamega Narayana Chendhooram* shows acidic pH.
- The $P^H$ level plays a role in enzyme activity by maintaining the internal environment, thus it exhibits an important role in regulating homeostasis.
- It is also an important factor for drug absorption [109]. Because of the acidic nature, the drug is more readily absorbed in an acidic medium like stomach which enhances the bioavailability of the drug.

**Loss on drying**

- Loss on drying (LOD) of *KMNC* gives the total amount of volatile content and moisture (water) present in the drug.
- The stability of a drug and its shelf-life are depends on moisture content.
- Moisture increase can adversely affect the active ingredient.
- Low moisture content- drug could get maximum stability and better shelf life.
- The low moisture content of *KMNC* indicates that it has long shelf life. Since the drug has low loss on drying (0.61%), the moisture content is less which is suitable for medicine.

**Ash values**

**Total Ash value**

High level of total Ash value of the trial drug *KMNC* contains (97.78%) indicates the richness of organic substances. These organic compounds are responsible for mineral supplements and therapeutic effect of *KMNC* and also it indicates it was under the process of incinerations.

**Acid insoluble ash**

Lower acid insoluble ash value (0.23%) better will be the drug quality [110]. The drug ensures a low value of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.

**Water soluble ash**

Water soluble ash value (4.76%) indicates the easy facilitation of diffusion and the osmosis mechanisms.

**Bio Chemical analysis:**

**Table No.10. Results of Basic radicals**

| Parameters | Result |
|---|---|
| Test for Potassium | Negative |
| Test for Calcium | Positive |
| Test For Magnesium | Negative |
| Test For Ammonium | Positive |
| Test For Sodium | Positive |
| Test for Iron (Ferrous) | Negative |
| Test For Zinc | Positive |
| Test For Aluminium | Negative |
| Test For Lead | Negative |
| Test for Copper | Negative |
| Test For Mercury | Positive |
| Test for Arsenic | Positive |

**Table No.11. Results of Acid Radicals**

| Parameters | Result |
|---|---|
| Test for Sulphate | Positive |
| Test for Chloride | Positive |
| Test for Phosphate | Negative |
| Test for Carbonate | Negative |
| Test for fluoride &oxalate | Negative |
| Test For Nitrate | Negative |

**DISCUSSION:**

- The Biochemical analysis for basic radicals of *KMNC* shows the presence of Calcium, Mercury, Arsenic, Sodium, Ammonium and Zinc .
- The Biochemical analysis for acidic radicals of *KMNC* shows the presence of Sulphate and Chloride.
- The Presence of these radicals helps *KMNC* for its therapeutic effect.

**Calcium:**

A randomized controlled trial found that 1400–1500 mg supplemental calcium and 1100 IU vitamin D3 reduced aggregated cancers with a relative risk. [111]

**Ammonium:**

Ammonia generated from the amino acid catabolism following glucose deprivation can also stimulate autophagy. This ammonia induced autophagy also promotes cell survival and thus represents a promising therapeutic target in cancer treatments.[112]

**Sodium:**

Sodium has a cytotoxic effect on cancer cells.[113]

**Zinc:**

Zinc is needed for its immune function, wound healing and blood clotting. Some experiments shows, that the Zinc slows the growth of cancer cells in the laboratory.[114]

**Mercury:**

Miles (1926) introduced perchloride of mercury as an antiseptic agent in rectal surgery. Goligher et al. (1951), Morgan (1955) and Keynes (1961) introduced the technique of flushing the colon and rectum in restorative cancer surgery. Perchloride of mercury solution was used as a anti-cancer agent in renal surgery. It is therefore concluded that mercury perchloride is a safe anti-cancer agent when it is used in a large bowel surgery. H Brendan Devlin et al.

**Arsenic:**

During the 18th and 19th centuries, a number of arsenic compounds were used as medicines. In that Arsenic trioxide has been used in a variety of method of treatment over the past 500 years, but most commonly used in the treatment of cancer. In 2000, the FDA approved this compound for the treatment of acute promyelocytic leukemia that is resistant to ATRA.

**Sulphate:**

Hydrazine sulphate is a chemical compound that has been studied as a treatment for cancer. Sulphate contains anti-cancer property.

**Chloride:**

Chloride has cyto toxic effects on cancer cells.[115]

**Availability of bacterial and fungal load in *Kaalamega Narayana Chendhooram***

**Table No.12. Bacterial and Fungal dilution:**

| Microbes | Dilution | Result |
|---|---|---|
| Bacteria | $10^{-4}$ | 1 |
| Bacteria | $10^{-6}$ | Nil |
| Fungi | $10^{-3}$ | Nil |
| Fungi | $10^{-2}$ | 2 |

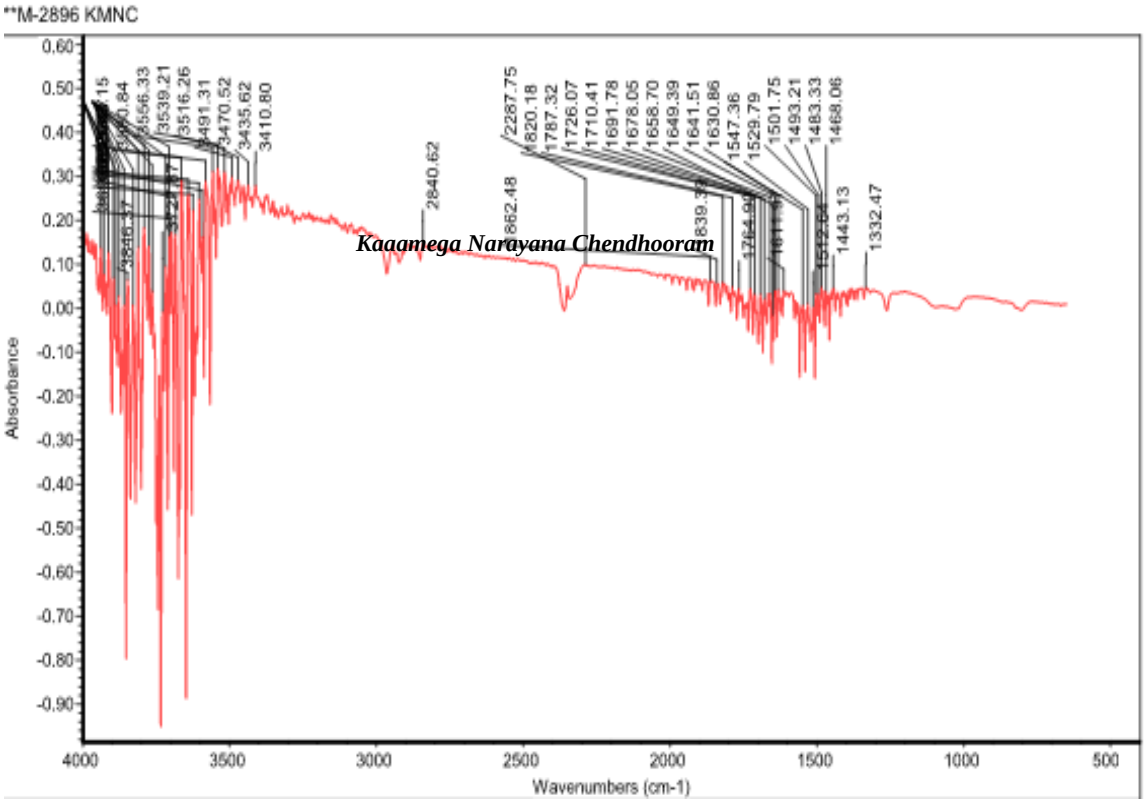
**DISCUSSION:**

- The availabity of bacterial load in the *KMNC* has been performed by plate count –Agar plate technique
- The microbial load have been analyzed for the micro-organisms contamination. The contamination of a trail drug by micro-organisms not only cause bio deterioration but also reduces the efficacy of drugs.
- The toxic effect produced by microbes makes the drugs to give no response for human consumption because the contaminated drug may develop several diseases instead of disease being cured.
- Here, the contamination of *KMNC* has been examined by bacterial and fungal load.
    - Total bacterial load in $10^{-4}$ dilution is 1 and in $10^{-6}$ dilution is nil.
    - Total fungal load in $10^{-2}$ dilution is nil and in $10^{-3}$ dilution is 2.

  Here, the contamination of *KMNC* is within the WHO norms. Hence, the drug is collected, prepared, stored and packed and decontaminated prior to formulation.

## INSTRUMENTAL ANALYSIS:

## FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY)




**Graph : 1 Peak values by FTIR**

| S.no | Absorption peak ($cm^{-1}$) | Stretch | Functional group |
|---|---|---|---|
| 1 | 3846 | O-H,H | Alcohols, phenols |
| 2 | 3727 | O-H,H | Alcohols, phenols |
| 3 | 3690 | O-H,H | Alcohols, phenols |
| 4 | 3580 | O-H,H | Alcohols, phenols |
| 5 | 3556 | O-H,H | Alcohols, phenols |
| 6 | 3539 | O-H,H | Alcohols, phenols |
| **7** | 3516 | O-H,H bonded | Alcohols, phenols |

| S.no | Absorption peak ($cm^{-1}$) | Stretch | Functional group |
|---|---|---|---|
| 8 | 3491 | O-H,H bonded | Alcohols, phenols |
| 9 | 3470 | N-H | 1°,2°amines, amides. |
| 10 | 3435 | O-H,H | Alcohols, phenols |
| 11 | 3410 | O-H,H | Alcohols, phenols |
| 12 | 2287 | H-C=O, C-H | Aldehydes |
| 13 | 1820 | C=O | α –β unsaturated aldehydes, Ketones. |
| 14 | 1862 | -C=C | Alkenes |
| 15 | 1820 | C=O | Carbonyl ( General ) |
| 16 | 1787 | C=O | Carbonyl ( General ) |
| 17 | 1764 | C=O | Carbonyl ( General ) |
| 28 | 1710 | C-O | α –β unsaturated aldehydes, Ketones |
| 29 | 1691 | C-O | α –β unsaturated aldehydes, Ketones |
| 30 | 1678 | -C=C | Alkene |
| 31 | 1658 | -C=C | Alkene |
| 32 | 1630 | N-H | 1°amine |
| 33 | 1611 | N-H | 1°amine |
| 34 | 1547 | N-O Asymmetric stretch | Nitro-compounds |
| 35 | 1529 | N-O Asymmetric stretch | Nitro-compounds |

| S.no | Absorption peak ($cm^{-1}$) | Stretch | Functional group |
|---|---|---|---|
| 36 | 1512 | N-O Asymmetric stretch | Nitro-compounds |
| 37 | 1501 | N-O Asymmetric stretch | Nitro-compounds |
| 38 | 1468 | C-H | Alkanes |
| 39 | 1332 | C-N | Aromatics amines |

**Table-13**

**Interpretation**

The wave numbers from 4000cm-1 to 1500cm-1 gives details for identification of functional group.

The wave number from 1500cm -1 to 400 cm-1 provides particulars about a molecular fingerprint.

The above result shows the presence of functional group like alcohols, Alkanes, amides in *Kaalamega Narayana Chendhooram*.

They may be responsible for the presence of anticancer action of *Kaalamega Narayana Chendhooram* in oral cancer.

**Amides**

Amide derivatives of Benzene- sulfonanilide, a Pharmaceutical composition is used in cancer treatment [111].The lead molecule of these compound was methane sulfonamide, a cyclo oxgenase (COX) inhibitor. They act as a efficient anti tumour agents[116].

**OH**

OH group of *KMNC* has higher potential towards inhibitory activity against microorganisms[117].

**Ketones:**

Ketones plays an important role in treating cancer cells. The eliminating carbohydrates can quickly lower calorie intake, reducing the energy available to the cells in the body. In turn, this may slow down the tumour growth and the cancer's progression[118].

**Aldehydes:**

Aldehydes plays an important role in maintain and differentiation of stem cells as well as normal development. Aldehydes are the potential therapeutic target for treatment of prostate cancer and also plays a key role in resistance to radiation therapy and tumour recurrence in prostate cancer[119].

**Nitro compounds:**

Nitro and nitroso compounds are potent, selective and nontoxic inhibitors, suppressants of cancer growth and viral infections. These compounds are particularly useful for the treatment and suppression of tumours and viruses.

**Phenols**

- The effect of phenols is currently of great awareness due to their anti carcinogenic activities.
- Phenolic acid components play an important role in the control of cancer and other human diseases.
- Phenols and flavanoids possess diverse biological activities, for example, antiulcer, anti-inflammatory, cytotoxic and antitumour, antispasmodic and antidepressant activities.

**Alkanes:**

Alkane derivative like bis (4-amino-5-mercapto-1, 2,4-triazol-3-yl) possess anti- cancer activity [120].

**Alkanes:**

Alkenes are the molecules containing a C=C double bond and is claimed to reduce the risk of heart disease and cancer.

**Carboxylic acid**

- Benzene-poly-carboxylic Acid Complex (BP-CI) is a novel anticancer complex against human cancer cells.
- Docosahexaenoic acid (DHA) is an omega-3 fatty acid. Its structure is a carboxylic acid (-oic acid) with a 22- carbon chain (docosa-is Greek for 22) and six (hexa-) cis double bounds [121].
- DHA was revealed to increase the efficacy of chemotherapy in prostate cancer cells and a chemo protective effect in a mouse model was reported.
- It may also be used as a non- toxic adjuvant to increase the efficacy of chemotherapy.
- In mice, DHA was found to reduce growth of human colon carcinoma cells
- The cytotoxic effect of DHA was caused by decrease in cell growth regulators.

**Ether:**

Certain ether lipids such as 1-0-octadecyl-2-0 methyl-rec-glycero-3-phosphocholine represent a new class of anti -neoplastic agents. These ether lipids have been shown to be cytotoxic for a wide variety of tumours.

**SEM**
**(SCANNING ELECTRON MICROSCOPE)**

The particle size and chemical elements were assessed by SEM is one of the most widely used instruments in research areas.

The following image is done by 10 K X magnification via 1µm aperture shows maximum depth focused.

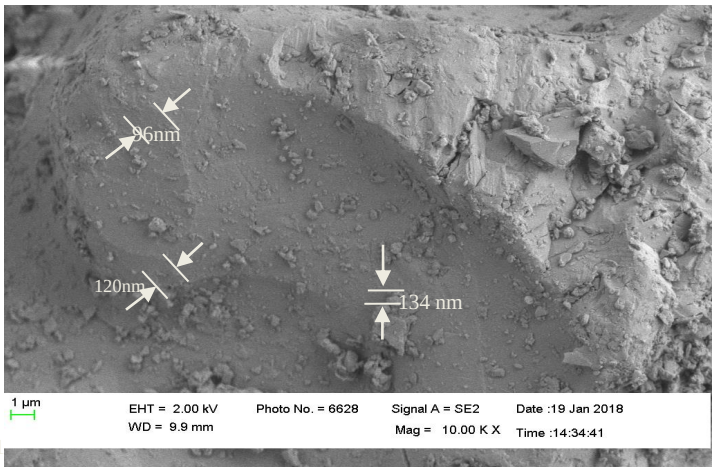



**Fig no: 29 Showing nano particles in SEM image of 1µm**

**Discussion on SEM reports**

- The above SEM study shows of microscopic resolution gives the ranges from 1, 20µm.
- The difference in morphology as evident from the micrograph is due to the presence of various substances in the sample.
- The presence of nanoparticles in a drug which are in size of 100nm has to be considered as a nanomedicine.

**Advantages of nano particles:**

- Enhancing solubility of hydrophobic drugs.
- Prolonging circulation time.
- Minimizing nonspecific uptake.
- Increasing therapeutic effect for the drug.
- Decreasing toxicity, side effects.
- Preventing undesirable side effects.
- Improving intracellular penetration.
- Improving stability and increased biavailability
- Specific cancer targeting [122].
- The test drug *Kaalamega Narayana Chendhooram* contains Nano particles.
- Nano particles present in the drug results in a better bioavailability and facilitates absorption. Thus they act on a cellular level and possess anticancer activity.

- Nanotechnology is a promising way from the cancer management towards cancer elimination.
- The particles of nano size shows that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase its therapeutic effect.
  The SEM images of *KMNC* shows the presence of particles are spherical in shapes, aggregated morphology.

The observed size of the particles are 96nm, 120nm, 134nm.The size of the particles are in and around nano range. This trial drug *KMNC* can be considered as nanomedicine. So, the bioavailability of the drug will be high. In addition to that the drug will be highly potent even in lower dose. Nowadays the research studies reveals that the current trends of cancer medications not only depends on chemotherapy, brachiotherapy, additionally it also needs a immunotherapy and especially nanotherapy. This nano medicine will helps to achieve magical remedy in the treatment of cancer in this modern World.

## ICP-MS RESULTS AND DISCUSSION

### ICP-MS interpretation of *KMNC*

**Table-14**

| S. no | Elements | Detected levels |
|---|---|---|
| 1. | Arsenic | BDL 2.465(below 3PPM) |
| 2. | Mercury | BDL 0.992(below 1PPM) |
| 3. | Lead | BDL (below 10PPM) |
| 4. | Cadmium | BDL (below 1PPM) |

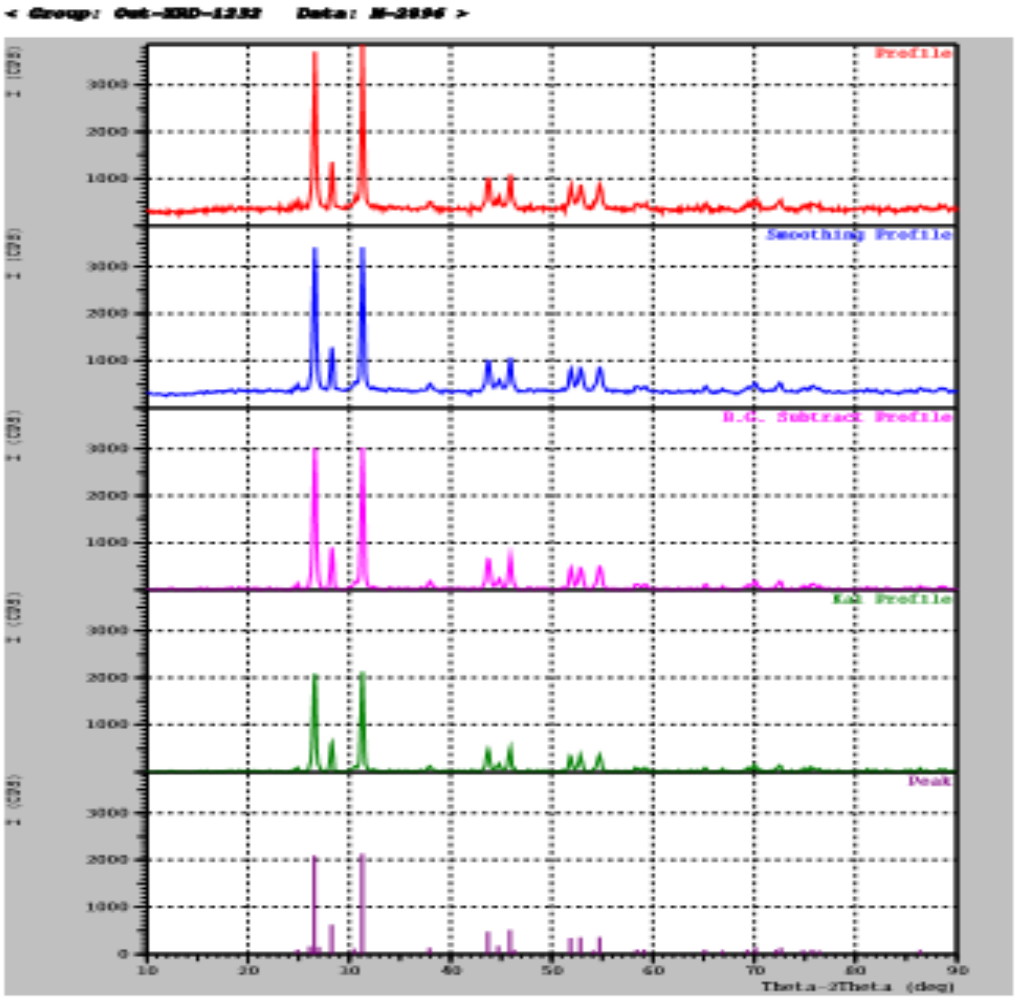
### Discussion on ICP-MS:

From the above results, that the presence of heavy metals Arsenic, Mercury are observed within the WHO permissible limits. Cadmium and lead are under the permissible limit. This ensures the safety of the drug for its therapeutic use and it will

give much more effectiveness in the treatment of diseases without causing any damages to the body cells. Safety of this drug apart from toxicological study, it is validated by heavy metal analysis through ICPMS as per WHO guidelines report.

## XRD (XRAY DIFFRACTION)

**Graph :2 XRD Interpretation**




Strongest 3 peaks of XRD analysis.

| No. | Peak | 2Theta |
|---|---|---|
| 1. | 7 | 31.2824 |
| 2. | 3 | 26.5708 |
| 3. | 5 | 28.2502 |

**Discussion**

The crystalline structure, the size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The nano particles may enhance the bio absorption of the drug.

XRD pattern of *Kaalamega Narayana Chendhooram* shows the good crystalline after incineration process. The major diffraction peaks are identified after XRD analysis *KMNC* concluded that in nano crystalline range (26-31nm) is association with organic molecules probably plays an important role in making its biocompatible and non toxic at its therapeutic doses. Other elements present in *KMNC* act as a additional supplement and possibly helps in increase the efficacy of the formulation [123]

**ACUTE ORAL TOXICITY**

**Dose finding experiment and its behavioral Signs of Toxicity for *Kaalamega Narayana Chendooram:***

**Observation done:**

**Table-15**

| SL | Group CONTROL | Observation | SL | Group TEST | Observation |
|---|---|---|---|---|---|
| 1 | Body weight | Normal | 1 | Body weight | Normal |
| 2 | Assessments of posture | Normal | 2 | Assessments of posture | Normal |
| 3 | Signs of Convulsion,Limb paralysis | Absence | 3 | Signs of Convulsion Limb | Absence of sign (-) |
| 4 | Body tone | Normal | 4 | Body tone | Normal |
| 5 | Lacrimation | Normal | 5 | Lacrimation | Absent |
| 6 | Salivation | Normal | 6 | Salivation | Normal |
| 7 | Change in skin color | No significant color change | 7 | Change in skin color | No significant color change |

| | | | | | |
|---|---|---|---|---|---|
| 8 | Piloerection | Normal | 8 | Piloerection | Normal |
| 9 | Defecation | Normal | 9 | Defecation | Normal |
| 10 | Sensitivity response | Normal | 10 | Sensitivity response | Normal |
| 11 | Locomotion | Normal | 11 | Locomotion | Normal |
| 12 | Muscle gripness | Normal | 12 | Muscle gripness | Normal |
| 13 | Rearing | Mild | 13 | Rearing | Mild |
| 14 | Urination | Normal | 14 | Urination | Normal |

**Table-16**

| No | Dose mg/kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1.** | **Control** | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| **2.** | **2000mg** | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - |

**Behavioural Signs of Toxicity for *KMNC***

1. *Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7.Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm 11. Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19 Respiration 20. Mortality*

## Table 17 ( Body weight Observation)

| DOSE | DAYS | | |
|---|---|---|---|
| | 1 | 7 | 14 |
| CONTROL | 280.2±42.30 | 281.4 ± 64.12 | 282.6 ±26.18 |
| HIGH DOSE | 279.4± 21.24 | 279 ± 3.64 | 279.4 ± 2 |
| P value (p)* | NS | NS | NS |

NS- Significant, **($p < 0.01$), *($p < 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

## Table: 18 (Water intake (ml/day) of Wistar albino rats group exposed to *Kaalamega Narayana Chendooram*

| DOSE | DAYS | | |
|---|---|---|---|
| | 1 | 7 | 14 |
| CONTROL | 61 ± 1.12 | 62±2.22 | 62.9±1.14 |
| HIGH DOSE | 61.2±1.1 | 61±1.14 | 61.20±24 |
| P value (p)* | NS | NS | NS |

N.S- Not Significant, **($p < 0.01$), *($p < 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

**Table 19: Food intake (gm/day) of Wistar albino rats group exposed to *Kaalamega Narayana Chendooram***

| DOSE | DAYS | | |
|---|---|---|---|
| | 1 | 7 | 14 |
| CONTROL | 56.24±2.22 | 56.2±7.42 | 57.4±3.46 |
| High DOSE | 56.6±1.63 | 55.6±2.62 | 55.1±5.38 |
| P value (p)* | NS | NS | NS |

N.S- Not Significant, **($p < 0.01$), *($p < 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

**Acute toxicity Discussion:**

In the acute toxicity study, the rats were treated with different concentration of *Kaalamega Narayana Chendooram* from the range of 5mg/kg to 2000mg/kg.

- This dose level did not produce the signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.
- However the behavior changes, Body weight, Water intake, food intake does not produce much significant, Thus the results are in non-significant.
- These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this drug.
- In acute toxicity test the *Kaalamega Narayana Chendooram* was found to be nontoxic at the dose level of 2000mg/ kg body weight.

## 28 DAYS OF REPEATED ORAL TOXICITY STUDY IN RATS

**Table 20: Body weight of wistar albino rats group exposed to *Kaalamega Narayana Chendooram***

| DOSE | DAYS | | |
|---|---|---|---|
| | 1 | 15 | 28 |
| CONTROL | 280.2±10.03 | 281.2 ± 10.24 | 281.6 ± 24.61 |
| LOW DOSE | 279.2 ± 40.20 | 279.5 ± 30.14 | 279.8± 52.40 |
| MID DOSE | 281.3±20.04 | 282.3±9.04 | 280±42.03 |
| HIGH DOSE | 279± 01.10 | 278.89 ± 10.30 | 279±04.32 |
| **P value (p)*** | NS | NS | NS |

NS- Not Significant, **(p < 0.01), *(p <0.05), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test.

**Table 21: Water intake (ml/day) of Wistar albino rats group exposed to *Kaalamega Narayana Chendooram***

| DOSE | DAYS | | |
|---|---|---|---|
| | 1 | 15 | 28 |
| CONTROL | 98.4 ± 1.25 | 98±1.02 | 98.8±2.30 |
| LOW DOSE | 97.2±6.40 | 97.4±8.50 | 97.6±1.14 |
| MID DOSE | 97.3±5.4 | 97.55±7.9 | 97.5±6 |
| HIGH DOSE | 97.8.1±1.30 | 97.20±2.20 | 97.7±4.52 |
| **P value (p)*** | NS | NS | NS |

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test.

**Table 22: Food intake (gm/day) of Wistar albino rats group exposed to *Kaalamega Narayana Chendooram***

| DOSE | DAYS | | |
|---|---|---|---|
| | 1 | 15 | 28 |
| CONTROL | 190.4 ± 2.25 | 191±7.14 | 191.4±4.20 |
| LOW DOSE | 190.2±1.12 | 189.2±1.02 | 189.4±4.14 |
| MID DOSE | 189.4±2.22 | 188.1±2.25 | 189±4.2 |
| HIGH DOSE | 188.1±1.20 | 188.2±2.21 | 188.6±2.40 |
| P value (p)* | NS | NS | NS |

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test.

**Table 23: Haematological parameters of Wistar albino rats group exposed to *Kaalamega Narayana Chendooram***

| Category | Control | Low dose | Mid Dose | High dose | P value (p)* |
|---|---|---|---|---|---|
| Haemoglobin(g/dl) | 10.2±0.24 | 10.10±0.36 | 10.1±0.11 | 9.28±0.26 | N.S |
| Total WBC ($\times 10^3$ l) | 14.52±0.05 | 14.42±0.13 | 14.44±012 | 14.40±6.16 | N.S |
| Neutrophils(%) | 26.15±0.01 | 25.11±0.22 | 25.23±1.13 | 26.20±2.30 | N.S |
| lymphocyte (%) | 79.10±1.06 | 79.23±1.02 | 78.24±0.23 | 78.26±4.46 | N.S |
| Monocyte (%) | 0.9±0.03 | 0.8±0.05 | 0.7±0.004 | 0.7±0.07 | N.S |
| Eosinohil(%) | 3.2±0.04 | 3.2±0.06 | 4.24±0.05 | 4.53±0.02 | N.S |
| Platelets cells$10^3$/µl | 604.16±2.66 | 600±4.26 | 599±3.32 | 599.06±4.54 | N.S |
| Total RBC $10^6$/ µl | 8.49±0.01 | 7.09±0.50 | 7.07±0.44 | 7.64±0.32 | N.S |
| PCV% | 37.65±0.6 | 37.30±1.32 | 37.44±1.44 | 37.66±2.24 | N.S |
| MCHC g/dL | 38.4±1.42 | 38.06±0.47 | 38.06 ±0.22 | 38.30±2.34 | N.S |
| MCV fL(µ$m^3$) | 54.04±4.60 | 54.06±3.43 | 54.05±3.23 | 54.34±2.14 | N.S |

N.S- Not Significant, **(p < 0.01), *(p <0.05),n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test).

**Table 24: Liver Function Test of Wistar albino rats group exposed to *Kaalamega Narayana Chendooram***

| Treatment | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **T BILIRUBIN (mg/dl).** | 0.50±0.07 | 0.52±0.16 | 0.54±0.44 | 0.60±0.15 |
| **Triglycerides (mg/dL)** | 66.24±7.74 | 66.14±6.22 | 65.12±5.33 | 65.14±09.32 |
| **Cholesterol (mg/dL)** | 66.61±4.45 | 66.10±6.46 | 65.02±0.44 | 62.14±2.43 |
| **T.Protein** | 7.14±0.12 | 6.84±0.24 | 6.87±0.22 | 5.36±0.42 |
| **SGOT/AST (U/L)** | 119.12±0.43 | 120.23±1.22 | 120.4±0.44 | 122±0.32 |
| **SGPT/ALT (U/L)** | 82.64±6.57 | 82.11±3.39 | 84.45±5.44 | 87.11±1.24 |
| **ALP (U/L)** | 215.08±10.48 | 228.40±11.68 | 232.6±0.99 | 236.44±10.94 |

NS- Not Significant, **($p < 0.01$), *($p < 0.05$), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

**Table 25 : Biochemical Parameters of of Wistar albino rats group exposed to *Kaalamega Narayana Chendooram***

| BIOCHEMICAL PARAMETERS | CONTROL | LOW DOSE | MID DOSE | HIGH DOSE | P Value (p)* |
|---|---|---|---|---|---|
| **GLUCOSE (R) (mg/dl)** | 108.63 ±0.81 | 111.08±0.9 | 110.04±0.3 | 108.3±0.47 | N.S |
| **T.CHOLESTEROL(mg/dl)** | 93.21± 1.16 | 93.8±1.50 | 93.3±1.65 | 93.03±1.15 | NS |
| **TRIGLY(mg/dl)** | 54.16±1.52 | 53.12±1.42 | 54.12±1.22 | 54.16±1.23 | N.S |
| **LDL** | 72.4±2.14 | 72.12±2.54 | 71.22±2.22 | 71.24±10.20 | NS |
| **VLDL** | 11.2±1.30 | 11.20±2.21 | 11.20±2.22 | 11.14±12.14 | NS |
| **HDL** | 27.14±6.12 | 27.42±2.30 | 27.33±2.34 | 28.17±2.14 | NS |
| **Ratio 1(T.CHO/HDL)** | 3.41±1.16 | 3.42±1.40 | 3.45±1.44 | 3.64±2.03 | NS |
| **Ratio 2(LDL/HDL)** | 1.92±1.14 | 1.91±1.12 | 1.91±1.23 | 1.96±08.02 | NS |
| **Albumin (g/dL)** | 5.43±0.16 | 5.50±0.52 | 5.23±0.56 | 5.42±9.48 | NS |

NS- Not Significant, **($p < 0.01$), *($p < 0.05$), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test

**Table 25: Renal function test of of Wistar albino rats group exposed to *Kaalamega Narayana Chendooram***

| PARAMETERS | CONTROL | LOW DOSE | MID DOSE | HIGH DOSE | P Value (p)* |
|---|---|---|---|---|---|
| **UREA (mg/dl)** | 26.70±0.19 | 26.50±0.26 | 26.6±0.22 | 27.68±1.24 | N.S |
| **CREATININE(mg/dl)** | 0.22±0.02 | 0.52±0.04 | 0.56±0.04 | 0.98±0.07 | N.S |
| **BUN(mg/dL)** | 21.10±0.20 | 25.16±0.90 | 22.3±0.33 | 29.14±1.22 | NS |
| **URIC ACID(mg/dl)** | 6.04±0.34 | 7.06±0.51 | 7.09±0.22 | 8.42±0.20 | N.S |

NS- Not Significant, **($p < 0.01$), *($p < 0.05$), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test).

**28 days of Repeated oral toxicity Discussion:**

- The dose selected for the 28 days of repeated oral toxicity study was 20mg,100mg ,200mg/kg of *Kaalamega Narayana Chendooram*
- All the animals were free of intoxicating signs throughout the dosing period of 28 days.

**Observations:**

Overall observations were similar in both sex rats.These studies were done with certain doses like low dose X ( 20mg), 5X (100mg), 10X ( 200mg).The values are non significant.

**Clinical signs of toxicity**

No clinical signs of toxicity were observed.There is a slight variations in the values but they are within the non significat ranges.

**Mortality**

No mortality was observed after 28 days repeated dose administration of *KMNC*. All animals were survived up to study termination period.

**Body weight**

There is a slight variations in the body weights when compared to their initial weight. No significant alterations were observed in body weight.

**Food and water consumption**

No effect of treatment was noted, they are within the non-significant ranges.

**Physiological activities**

There is no changes in their general behavior.

**Blood analysis**

a. **Hematology**

   No treatment related effects were observed. However there is a slight variations in the result but they were within the permissible limit.

**b. Biological parameters**

There is a slight difference has been noted but it is normal within the normal limit. No treatment related effects were observed.

**c. Histological examination**

Histological examination of organs did not show as much pathological variations.

**Discussion**

- The acute and repeated 28 days oral toxicity studies of *KMNC* did not produce any toxicity signs in wistar albino rats. Daily administration of *KMNC* at different doses 50mg/kg, 100mg/kg for 28 days was tolerated by the rats without any mortality and morbidity, indicates the drug tolerance.
- There was a slight changes were observed in hematological report
- Hence the metalo - mineral formulation of *KMNC* can be considered to be safe drug for prolonged use as revealed by toxicological studies.

**HISTOPATHOLOGY EXAMINATION:**

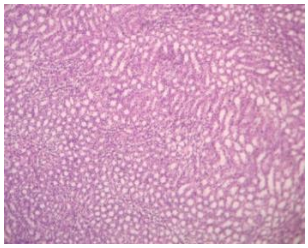
- Histopathology studies were carried out on liver, kidney and spleen and recorded. Blood samples for hematological and blood chemical analyses were taken from common carotid artery.

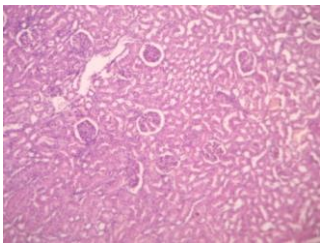
- All rats were sacrificed after the blood collection. The internal organs and some tissues were observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

## Fig no : 30 HISTOPATHOLOGY SLIDES:

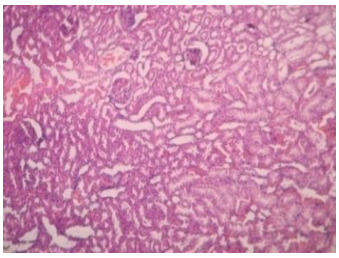
**Control:** **High dose of *KMNC* :**

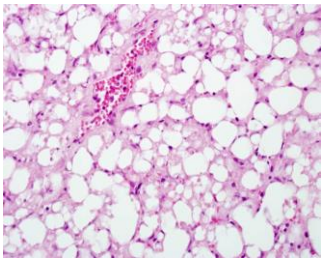
**Kidney**



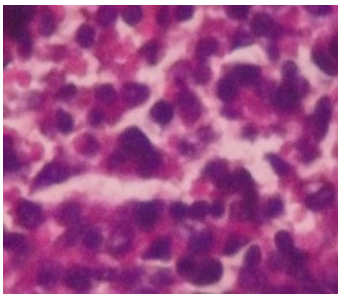


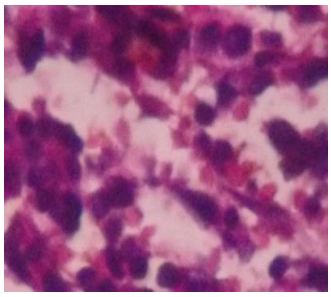
**Liver**





**Spleen**





## Discussion on Histopathology:

- From the histopathological examination, the slides of animal's organ didn't reveal abnormalities.
- From the acute and 28 days of repeated oral toxicity studies shows some significant changes were observed. But the values were found within the normal limits. So the drug *KMNC* was non-toxic and safe. So the drug KMNC is considered to be no observed adverse effect level ( NOAEL ) drug.
- Thus the safety of the drug is revealed so that it can be administered for long time without side effects.

# PHARMACOLOGICAL STUDY

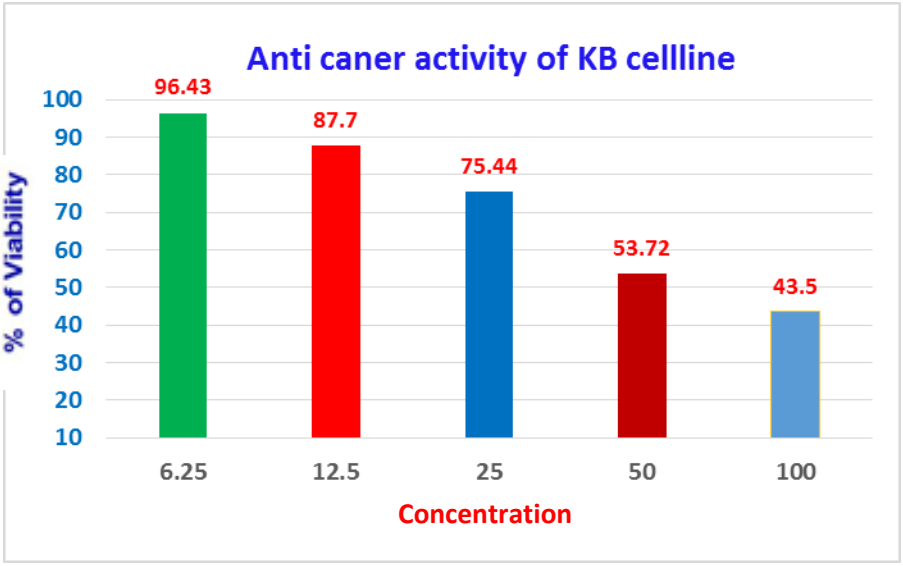
## Anti-cancer activity CELL LINE: KB Cell line

**Table-26**

| **Sample Concentration (µg/mL)** | **OD value I** | **OD value II** | **OD value III** | **Average OD** | **Percentage Viability** |
|---|---|---|---|---|---|
| **Control** | 0.3859 | 0.3922 | 0.3642 | 0.3808 | 100 |
| **Sample code: *KMNC*** | | | | | |
| 6.25 | 0.3647 | 0.3666 | 0.3703 | 0.3672 | 96.43 |
| 12.5 | 0.3606 | 0.3102 | 0.3311 | 0.3340 | 87.70 |
| 25 | 0.2808 | 0.2986 | 0.2824 | 0.2873 | 75.44 |
| 50 | 0.2277 | 0.1891 | 0.1969 | 0.2046 | 53.72 |
| 100 | 0.1626 | 0.1685 | 0.1659 | 0.1657 | 43.50 |

\

**IC50 Value : *KMNC shows the 50 % inhibitory concentration at 50*µg/mL**




**Graph: 3 KB cell lines.**

Graph -3 shows the drug dose and % of Inhibition of KB Cell line after treating with *KMNC*. It can be observed by the result of MTT assay that the IC dose of *KMNC* is 50µg/ml. As the dose increases the KB cell viability decreases. It was found that the % of growth inhibition increasing with increasing concentration of *KMNC* steadily up to 6.25 µg/ml on KB line (Table-26, graph -3) and that IC value on *KB cell* line was 50 and R value was 0.3808.

***Cytotoxicity Assay by MTT***

MTT colorimetric method, is a method for detecting cell survival and growth methods. This assay is based on the metabolic reduction of 3- (4,5- dimethylthiazol-2-yl) -2,5-difeniltetrazol (MTT) by mitochondrial enzyme succinate dehydrogenase in a colored compound blue (formazan), allowing to determine the functionality of the mitochondrial treated cells. This method has been widely used to measure survival and cell proliferation. The amount of living cells is proportional to the amount of formazan produced. Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination.

*KMNC* at different doses (6.25-100 µg in100 µl of 5% MEM) was administered for 24 hrs. It was found that the number of cells decreases as the dose increases and at approximately 50 µg/ml dose of extract, 50% of the cells (KB cells) were less as

compared to normal control as shown in graph no (3). The percentage of cells viability was determined by calculating the O.D of treated against the control. Reading optical density (OD) is performed in a spectrophotometer at a wavelength of 540 nm. Comparison values are made on a basis of 50% inhibition of growth ($IC_{50}$) in treated cells with specific agents. Results are tabulated in Table (25) and graphically represented in Graph(3).

***Analysis of Membrane Morphological Characteristics by Haematoxylin /Eosin (H/E) Staining***

Morphological changes such as changes to the cell membrane, loss of membrane asymmetry and cell shrinkage, are the early stage of apoptosis was analyzed by H/E staining. The IC dose (50µg/ml) treated cancer cells show features of apoptosis whereas treated with same amount of dose, to normal treated cells appeared without any significant changes.

- Since the discovery of the Cisplatin anti-tumour activity, great efforts have focused on the rational design of metal-based anticancer agents that can be potentially used in cancer chemotherapy.
- Over the last four decades, a large number of metal complexes have been extensively investigated and evaluated *in vitro* and *in vivo* [124].
- The key focuses of these studies lie in finding novel metal complexes which could potentially overcome the hurdles of current clinical drugs including toxicity, resistance and other pharmacological deficiencies.
- Metalo-mineral and Minerals compounds have been used in medicine for several thousands of years.
- The medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance. Monographs and major reviews, as well as dedicated volumes, testify to the growing importance of the discipline [125].
- The field of inorganic chemistry in medicine may usefully divided into two main categories: firstly, ligands as drugs which target metal ions in some form, whether free or protein-bound; and secondly, metal-based drugs and imaging agents where the central metal ion is usually the key feature of the mechanism of action.

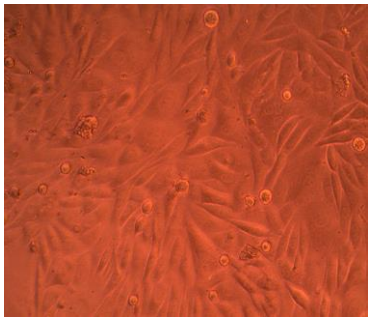
- Arsenic trioxide, $As_2O_3$ (Trisenox, Cell Therapeutics Inc, Seattle,USA) which was approved by the FDA in September 2000 for the treatment of Acute Promyelocytic Leukemia (APL) in patients who have relapsed or are refractory to retinoid and anthraycline chemotherapy.
- An estimated 1,500 new cases of APL are diagnosed yearly in the US, of which an estimated 400 patients will not respond to, or will relapse from, first-line therapy .
- The approval of arsenic trioxide as a chemotherapeutic agent invokes the pioneering work of Ehrlich and the development of Salvarsan for use in syphilis- the foundation stone for the science of chemotherapy [126].
- The use of chelating agents in medicine may even be traced to a collaboration between Werner (the father of coordination chemistry) and Ehrlich (the father of chemotherapy) to find less toxic arsenic compounds for the treatment of syphilis [119].
- Arsenic has been used therapeutically for more than 2,000 years and was used in the 1930s for treatment of chronic myelogenous leukemia until by the development of newer chemotherapies.
- The past, present and future of medicinal arsenic has been described as a story of ‘‘use, dishonor, and redemption’’.
- Recent interest in arsenic trioxide initially arose through Chinese reports of its efficacy and use. Side effects are cardiotoxicity, skin rashes and hyperglycemia [127].
- Arsenic trioxide apparently affects numerous intracellular signal transduction pathways and causes many alterations in cellular function.
- Thus, the mechanisms of cell death induced by arsenic trioxide are multiple; inductions of apoptosis, inhibition of proliferation and even inhibition of angiogenesis have all been reported.
- In cellular studies, arsenic trioxide inhibits glutathione peroxidase, possibly through generation of arsenic–GSH conjugates, and increases cellular hydrogen peroxide content [128].
- The incineration process of the Mercury and Sulphur macro particles are became very smaller and this may be possible for devoid of toxicity and more potent in Anticancer therapeutic.

- Arsenic disulfide, a major effective component of realgar, has been investigated for its anti-cancer potential and shown to have therapeutic efficacies in hematological and some solid tumours.

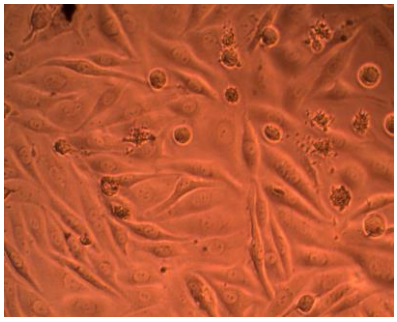
- Several pieces of evidence indicate that iron deprivation could be an excellent therapeutic approach:
  (i) dietary iron restriction markedly decreases tumour growth in rodents
  *(ii)* The antibodies which block transferrin-binding to cellular receptors inhibit cancer cell growth *in vitro* and *in vivo* [129]
- Sulphur is commonly used in Asia as a medicine to treat inflammation and cancer.
- Sulphur has both the diol-containing compounds, 2a and 3, were the most cytotoxic of the sulfide series against V-79 cells in vitro (IC (90) = 2.1 micro M and 1.9 micro M, respectively). A preliminary anticancer screening against P388 leukemia showed that 2a is highly active in vivo as well
- Organic sulphur has been studied on oral and other cancers and has been found to have remarkable benefit in anti-cancer therapy .
- Oncologists and scientists engaged in the research of cancer treatments should conduct a comprehensive study on the efficacy of mercury which is being used as an anti-cancer drug in the age old Siddha system [130].
- Three years of research has shown that metal (Mercury, Arsenic and Copper) based Siddha drug is a safe alternative for Cisplatin therapy or arsenic trioxide in selected cases of cancer treatments wherein the patients cannot bear the adverse effects. The mice treated with Siddha drugs showed better health,what did in cisplatin therapy in terms of appetite, haemoglobin, red blood cells and white blood cells.
- Thus from the above study, it is evident that the cytotoxic property of *Kaalamega Narayana Chendhooram* may be due to the synergistic interactions between the metal complex.

**KB Cell lines:**

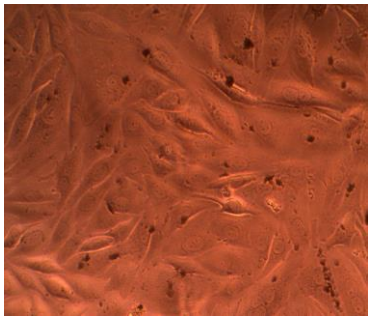
**Fig no: 30. Cancer cells in treated with various concentrations of *KMNC*:**



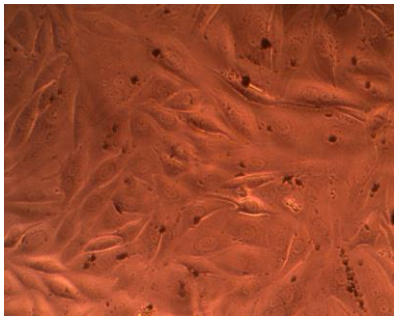
**Control**



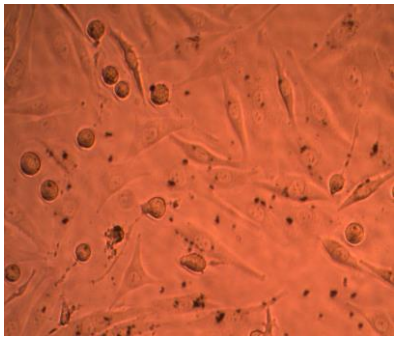
***KMNC* treated with 6.25 µg/mL**



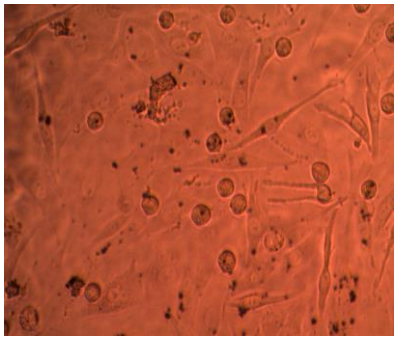
***KMNC* treated with 12.5 µg/mL**



***KMNC* treated with 25 µg/mL**



***KMNC* treated with 50 µg/mL**



***KMNC* treated with 100µg/mL**

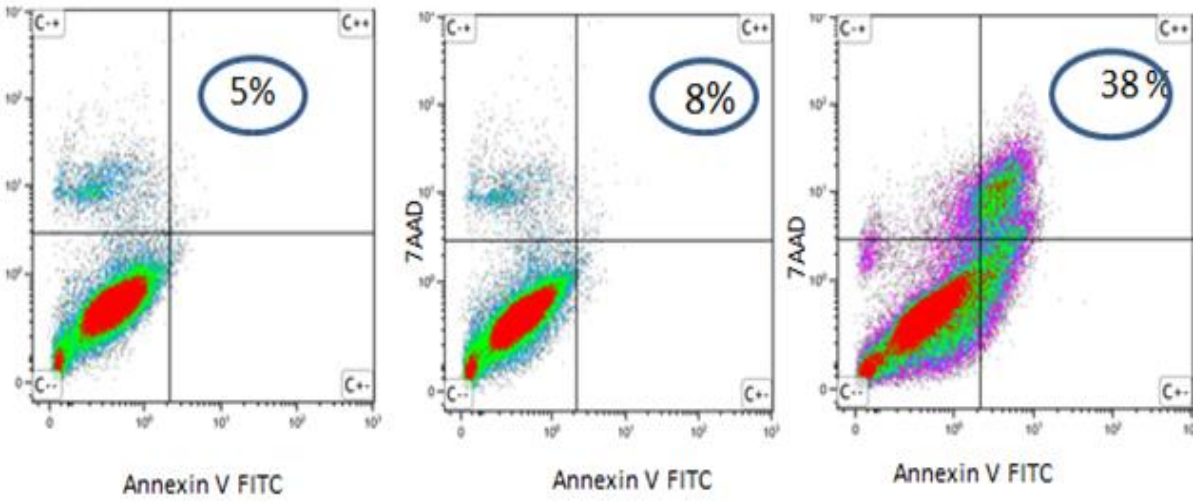
## RESULT AND DISCUSSION:

### Anti-Tumour Activity:

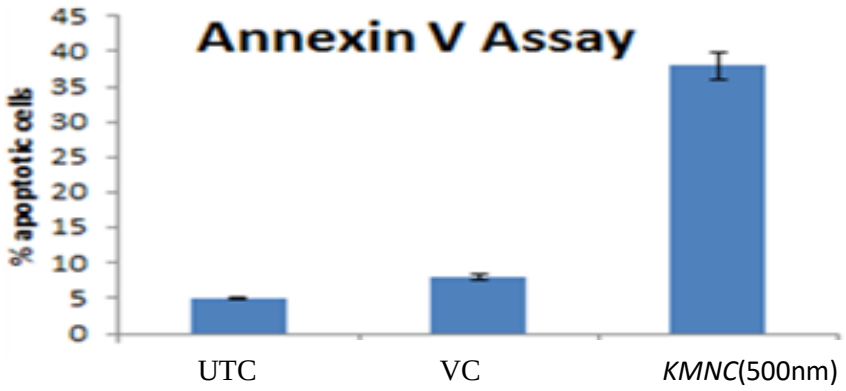
### Apoptosis Assay

To verify the results of cell viability assays, Annexin V and propidium iodide (PI) double staining was used to quantify apoptosis. AMOS III cells were either treated with *Kaalamega Naarayana chendhooram* at 500 nm for 48 hours. Cells were labeled with Annexin V–FITC conjugate and PI using the Annexin V assay kit following the manufacturer's instructions (Sigma, St Louis, MO) and analyzed using the BD Cell Quest Pro software. These results were further verified using Western blot analysis for specific caspases and Poly (ADP-ribose) polymerase (PARP) assay.

**UNTREATED CELL** **VEHICLE CELL** ***KMNC* (500nm)**

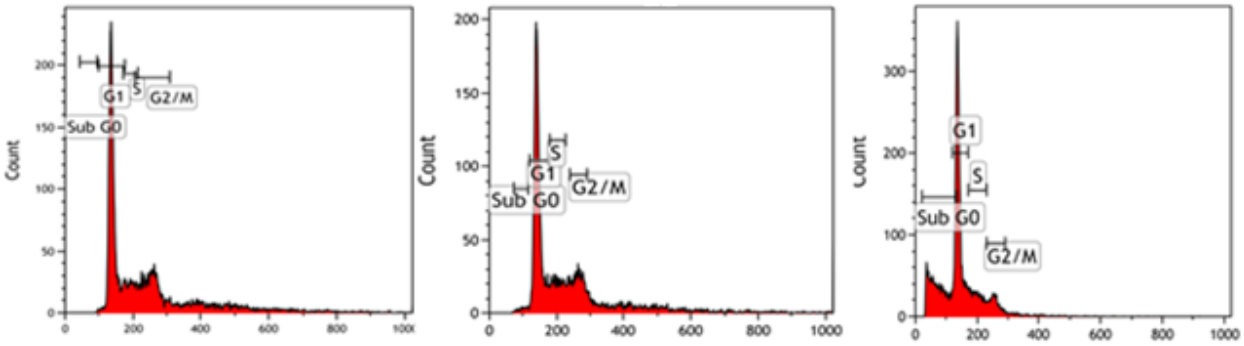



**Graph no:4**

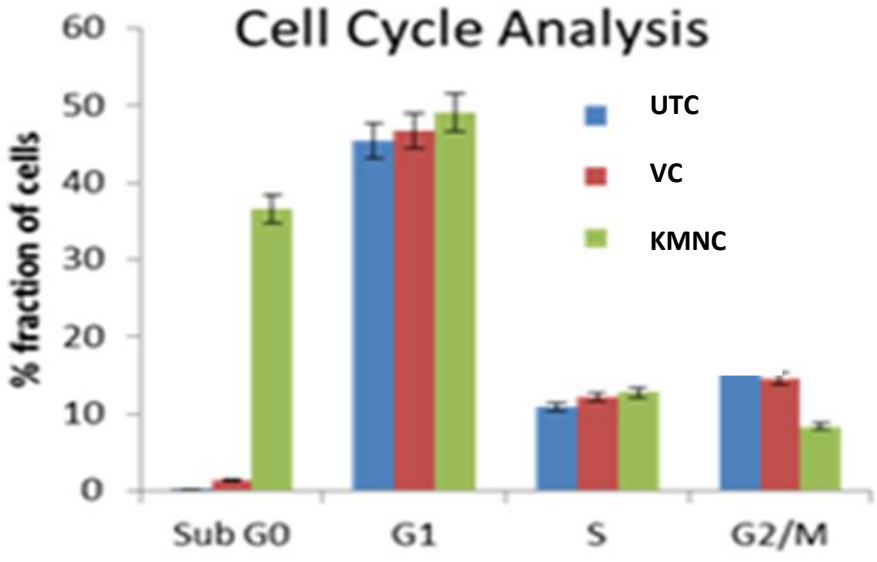



**Graph : 5**

**UNTREATED CELSS** **VEHICLE CONTROL** ***KMNC* (500nm)**




**Graph no :6**




**Graph no:7 .** ***Annexin V assay and Cell Cycle analysis.***

***(A)*** *Kaalamega Naarayana Chendhooram treated AMOS III cells showed significant increase in early and late apoptosis (38%) as compared to the untreated (5%) and vehicle control cells (8%) by annexin V assay.*

***(B)*** *Kaalamega Naarayana Chendhooram treated AMOS III cells show significantly higher number of cells in the Sub $G_o$ phase and $G_2M$ phases as compared to the untreated control AMOS III cells.*

**DISCUSSION**

In this study, we investigated the anti-proliferative and apoptotic activities of *KMNC* on human OSCC cell lines. The present study showed that *KMNC* has potential time and dose dependent anti-proliferative effect on OSCC cancer cell lines. We found that IC for *KMNC* was 38% for OSCC cell lines. As shown by the flow cytometry results, when the concentration of *KMNC* was increased, the percentage of early apoptotic cells also increased. For this reason, the mode of cell death appears to be due to early apoptosis cell death pathway.

**RESULT AND DISCUSSION:**

**Antimicrobial activity:**

**Antimicrobial activity of *Kaalamega Narayana Chendooram***

**GRAM NEGATIVE BACTERIA.**

**1. Table: 28 *E.coli***

| **Sample** | **Concentration (µg/mL)** | **Zone of inhibition (mm)** |
|---|---|---|
| **KMNC** | Streptomycin (100µg) | 20 |
| | 250 | 15 |
| | 500 | 16 |
| | 1000 | 19 |

**14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive**

**2. Table: 29 *Pseudomonas aeroginosa***

| Sample | Concentration (µg/mL) | Zone of inhibition (mm) |
|---|---|---|
| **KMNC** | Streptomycin (100µg) | 24 |
| | 250 | 19 |
| | 500 | 20 |
| | 1000 | 23 |

**14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive**

**3. Table: 30 *Klebsiella pneumoniae***

| Sample | Concentration (µg/mL) | Zone of inhibition (mm) |
|---|---|---|
| **KMNC** | Streptomycin (100µg) | 22 |
| | 250 | 17 |
| | 500 | 19 |
| | 1000 | 23 |

**14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive**

## GRAM POSITIVE BACTERIA

**4. Table: 31 *Staphylococcus aureus***

| Sample | Concentration (µg/mL) | Zone of inhibition (mm) |
|---|---|---|
| **KMNC** | Streptomycin (100µg) | 28 |
| | 250 | 20 |
| | 500 | 27 |
| | 1000 | 29 |

**14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive**

**5. Table: 32 *Streptococcus mutans***

| Sample | Concentration (µg/mL) | Zone of inhibition (mm) |
|---|---|---|
| **KMNC** | Streptomycin (100µg) | 24 |
| | 250 | 20 |
| | 500 | 22 |
| | 1000 | 24 |

**14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive**

**Note: Concentration of stock 10mg/mL DMSO**

**Inference:**

1. *Streptococcus mutans* - Highly sensitive in 500 (µg/mL)

2. *Staphylococcus aureus* - Highly sensitive in 250 (µg/mL)

3. *Escherchia.coli* - Highly sensitive in 250(µg/mL)

4. *Klebsiella pneumoniae* - Highly sensitive in 250 (µg/mL)

5. *Pseudomonas aeruginosa* - Highly sensitive in 250(µg/mL)

**Discussion:**

The development of resistance against the presently available antibiotics arises the necessity of rediscovery of new anti-bacterial agents in traditional systems of medicine. Different dosages of test drug against the microbes in antimicrobial activity of *KMNC* was compared with Standard drug Streptomycin (100µg)/ml disc for the following pathogens, they are *Escherchia.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus mutans*. The results represents *KMNC* potencially inhibit the growth of all above organism in 250µl, 500µl and 1000µl / disc. 14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive. The findings reveal that the Siddha drug *KMNC* have anti microbial potency against bacterial pathogens which is used in the treatment of diseases. The results of a trail drug will build a challenging weapon to achieve its antimicrobial activity in malignant tumours.

**Conclusion:**

Cancer is one of the most dreadful disease and have an increasing incidence in recent years. There is a huge need in finding a permanent cure for oral cancer. Hence I derived a new formulation based on the basic principles of *Siddha* system of medicine. Rather, the destructive process can be reversed by adapting the time tested higher order Siddha medicine loaded with positive. Hence I have preferred to choose the higher order medicine "*KMNC*" in treating cancer as per Siddha classical literature. *Siddha* formulation *KMNC* has proved its efficacy in preliminary physico-chemical analysis, instrumental analysis and in vitro anticancer assay. . *Kaalamega Naarayana Chendhooram* showed promising anticancer activity in oral cancer cell lines killing cancer cells by apoptosis. Further, *Kaalamega Naarayana Chendhooram* demonstrated promising anti-tumour activity in oral cancer tumour xenografts without significant toxicity to normal tissues underscoring the pre-clinical efficacy of *Kaalamega Naarayana Chendhooram*, as a potential anti-cancer therapeutic for oral cancer management. *Kaalamega Naarayana Chendhooram* also shows a resistance aginst the microbes which causing cancer. From the bird view, I would like to explore that the trail drug *KMNC* plays a major role in treating cancer cells in cell lines, anti tumour effect and anti-microbial effect.

# 6. CONCLUSION

Oral cancer is the sixth most common malignancy found worldwide and some are highly resistant to radiotherapy. The chemotherapy drugs also deliver intolerable side effects which are worse than the disease. This paved way for a novel anticancer drug which cures Oral cancer in a non-invasive way.

The intention of this study is to provide a solution for the above need. For a non-violent anticancer drug to Oral cancer, as a trial drug ***"Kaalamega Narayana Chendhooram"*** was taken from the classic Siddha Literature ***Athmaraksha mirtham Ennum Vaithiya Saara Sangeraham*** written by ***Kandhasamy Mudhaliyaar*** which was categorized by the department of AYUSH as a classical text.

Throughout the study, the safety and efficacy were tested thoroughly. The procedure for drug preparation and its techniques for standardization revealed GMP.

The trial drug *KMNC* has satisfied all parameters of testing protocol for *Chendhooram* which was assigned by AYUSH. It showed the accurate production and potency of "*Kaalamega Narayana Chendhooram"*

Physico-chemical analysis revealed better bio-availability and richness of its mineral content. Favouring this study were the presence of inorganic matters which were found through experiments for analyzing acid and basic radicals.

Various instrumental analysis of "*Kaalamega Narayana Chendhooram"*such as FT-IR spectroscopy, X-ray diffraction and scanning electron microscope demonstrated its chemical constituents, functional groups and particle size to support its indication to counter oral cancer

Under OECD guidelines, the acute and 28 days repeated oral toxicity studies proved the safety of "*Kaalamega Narayana Chendhooram"*at particular dose level. It is very useful in therapeutic dose determination.

The pharmacological activities are justified by anticancer effect on KB cell lines, anti-tumour effect on OSCC cell lines and anti-microbial activity. The anti-microbial activity of trial drug was also considered for its potential.

Factors like safety, efficacy, long self like, bio-availability, presence of significant elements, anions and cations and minerals favouring the activity justifies the main perspective of this study.

*KMNC*'s anti-cancer effect could be validated scientifically. Due to its Non-toxic anti-cancer effect, it would benefit the health community and the world.

# 7. SUMMARY

The validation of therapeutic efficacy of the trial drug **"*Kaalamega Narayana Chendhooram"*** was taken from the classic Siddha Literature ***Athmarakshamirtham Ennum Vaithiya Saara Sangeraham*** written by ***Kandhasamy Mudhaliyaar*** for oral Carcinoma *(Kanna Putru),* anti-tumour, anti-microbial activities were dealt with in the entire study.

- Introduction of the study comprises about the Siddha concept, Modern concept, prevalence of Oral Carcinoma worldwide, Complications of modern treatments which is found to be the major cause of the death in human and the role of the test drug in treating Oral cancer.
- Review of literature under various categories was carried out. It was elaborated under *Gunapadam* and Modern aspect of ingredients, Siddha and modern aspect of the disease, pharmaceutical aspect and pharmacological aspect in both Siddha and modern.
- All the ingredients were identified and authenticated by experts.
- The drug was subjected to analysis such as physiochemical, biochemical and instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug.
- The sample was also analyzed for anti-microbial activity to ensure its accuracy.
- For the study protocol, required animals were approved by the IAEC under CPCSEA.
- Toxicological study was made according to OECD guidelines comprising both acute and repeated oral dose 28days toxicity studies in wistar albino rats. It showed the safety of the drug which attributes its utility in long time administration.
- Pharmacological studies were completed. It revealed the anti-cancer, anti-tumor and anti-microbial activities of ***"****Kaalamega Narayana Chendhooram"*.
- Results and discussion gives the essential validations to prove the potency of the drug.
- Conclusion gives a Compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

# 8. FUTURE SCOPE

Trial drug for the study **"*KAALAMEGA NARAYANA CHENDHOORAM* "**was taken from the classic Siddha Literature ***Athmaraksha mirtham Ennum Vaithiya Saara Sangeraham*** written by ***Kandhasamy Mudhaliyaar***. Its validation for its Anti-cancer nature was completed at preliminary level. The result enhanced and assured its Anti-cancer property against oral cancer. More specific experiments on animal models and also clinical trials are required to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of oral cancer and satisfy the safe and painless anti-neo plastic treatment.

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# The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

*This Certificate is awarded to Dr/Mr/Mrs.....R. ABINAYA.....*

*For participating as ~~Resource~~ ~~Person~~ / Delegate in the Twentieth Workshop on*

**"RESEARCH METHODOLOGY & BIOSTATISTICS"**

*For **AYUSH Post Graduates & Researchers***

*Organized by the Department of Siddha*

*The Tamil Nadu Dr. M.G.R. Medical University From 07th to 11th March 2016.*

**Dr.N.KABILAN,** M.D.(S)
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NATIONAL SEMINAR ON

# "RESEARCH METHODOLOGY AND PUBLIC HEALTH INITIATIVE THROUGH SIDDHA SYSTEM OF MEDICINE"

## (RM & PHISSM – 2018)

6TH & 7TH APRIL 2018

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सिद्ध क्षेत्रीय अनुसन्धान संस्थान
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**SIDDHA REGIONAL RESEARCH INSTITUTE**
Poojappura, Thiruvananthapuram, Kerala

केन्द्रीय सिद्ध अनुसन्धान परिषद्
(आयुष मंत्रालय, भारत सरकार)
**CENTRAL COUNCIL FOR RESEARCH IN SIDDHA**
Ministry of AYUSH, Govt. of India

This is to certify that Dr./Shri/Smt. Abinaya R., GSMC Chennai has ~~participated~~/presented a paper entitled Scientific Validation of Standardization of a Novel Siddha Drug "Kaalamegha Narayana Chendooram" through the Modern Techniques. in the National Seminar on "Research Methodology and Public Health Initiative through Siddha System of Medicine" (RM & PHISSM – 2018) organized by Siddha Regional Research Institute, Thiruvananthapuram on 6th & 7th April 2018 at Dr. M R DAS Convention Centre, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala.

डॉ. ए. कनगराजन / Dr. A. Kanagarajan
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RM & PHISSM 2018

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WORKSHOP ON

**TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS**

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This is to certify that Dr./~~Mr.~~/~~Ms.~~ R. ABINAYA

of Govt. Siddha Medical College, Chennai has participated in the two-day workshop on "**TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS**" organized by the Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Chennai during 31st January – 1st February 2018.

**Chair Person & Coordinator**
**Dr. B. SHEELA RANI**
**Director (Research)**

**Convener**
**Dr. R. SELVARAJ**
**Scientist In-charge**

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# Certificate of Achievement

This Certificate is proudly presented to

Dr. Abinaya. R
GSMC, Chennai

for making oral presentation/poster presentation titled

Management of Cancers with a novel Siddha Formulations

in the National Conference on "*Prevention and Management of Lifestyle Disorders through Siddha system of Medicine*" on **the first Siddha Day** held on **04.01.2018** – organised by Central Council for Research in Siddha (CCRS) jointly with Directorate of Indian Medicine and Homoeopathy, Govt. of Tamil Nadu, The Tamil Nadu Dr. M.G.R. Medical University and National Institute of Siddha.

Prof. Dr. R. S. Ramaswamy
(Director General
Central Council for Research in Siddha)
Chairman

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ஆயில்யம்
சித்த மருத்துவத்
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(Joint Director, DIM&H
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IN COLLABORATION WITH

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सत्यमेव जयते

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# 1ST INTERNATIONAL CONFERENCE & EXHIBITION ON SIDDHA MEDICINE -2018

23RD - 27TH AT UNIT OF SIDDHA MEDICINE
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SRI LANKA

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THIS IS TO CERTIFY THAT Prof / Dr. / ~~Mr.~~ / Ms. ....R. ABINAYA..... ~~PARTICIPATED~~ / PRESENTED A PAPER ON ..STANDARDIZATION OF A NOVEL SIDDHA DRUG "CHANDAMAARUTHA "CHENDTHODRAM" THROUGH CHARACTERIZATION BY PHYSICO-CHEMICAL ANALYSIS & FOURIER TRANSFORM INFRARED RADIATION(FTIR) ANALYSIS..... IN THE ABOVE CONFERENCE HELD ON 26TH & 27TH FEBRUARY 2018.

Prof . R. VIGNESWARAN
VICE CHANCELLOR
UNIVERSITY OF JAFFNA

Shri. A. NATRAJAN
CONSULATE GENERAL,
HIGH COMMISSION OF INDIA
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# GOVERNMENT SIDDHA MEDICAL COLLEGE

Arumbakkam, Chennai, 600106

This certificate is awarded to Dr./~~Mr./Ms.~~ R. ABINAYA

for participating as a ~~resource person~~ / delegate in the seminar on

## "Orientation to research Methods"

Organised by Suzhumunai Scientific forum Government Siddha Medical College on 22 March 2018

**Dr. P. Manickam**
Scientist E
(ICMR) National Institute of Epidemiology

**Dr. K. Kanakavalli**
Principal
Govt. Siddha Medical College

*One day National Seminar on*

# "ROLE OF NEURO INFLAMMATORY MEDIATORS IN NEURODEGENERATIVE CONDITIONS IN DRUG DISCOVERY"

*Conducted by*

## Department of Pharmacology
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This is to Certify that Dr/ Mr/Ms. R. ABINAYA

has Participated as Resourse person / Chair person / Delegate ✓ in the Seminar in ***"Role of neuro inflammatory mediators in neurodegenerative conditions in drug discovery"*** on 24th January 2018

**Dr.R.PRAKASH**
Organizing Secretary

**Dr.V.VAIDHYALINGAM**
Director

**Dr.A.MEENA**
Convenor

# A COLLOQUIUM ON
# NEERARITHALIL NOI ARITHAL

## (நீரறிதலில் நோயறிதல்)

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*has participated in the colloquium on* NEERARITHALIL NOI ARITHAL *held*

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**Dr. P. MANICKAM** B.S.M.S., M.Sc., (Epidemiology)
TECHNICAL ADVISER
PAANIGAI

**Dr. G. SHANKAR** B.S.M.S.,
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# A COLLOQUIUM ON CLINICAL SIDDHA MEDICINE

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PAANIGAI 2016

## Certificate

*This is to certify that Dr.* ABINAYA. R

*has participated in the colloquium on* CLINICAL SIDDHA MEDICINE

*held at M.G.R. Janaki College of Arts & Science for Women, Raja Annamalai Puram, Chennai*

*On 12 - 06 - 2016 Sunday*

**Dr. V. BALAMURUGAN** B.S.M.S.,
TECHNICAL ADVISER
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**Dr. G. SHANKAR** B.S.M.S.,
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# THARU
## SIDDHA MARUTHUVA PAYILAGAM

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# Certificate of Participation

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Organized by Tharu-Siddha Maruthuva Payilagam

(Unit of Sai Siddha Foundation) at World University Service Centre, Chennai

on 31st July, 2016

Dr. G.Sankar, B.S.M.S.
Patron

Dr. G. Sivaraman, B.S.M.S., Ph.D.,
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Government of India
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# Certificate of

## Participation

This Certificate is proudly presented to

Dr. Shri. Abinaya.R [GSMC, Chennai]

for participating

in the National Conference on "*Prevention and Management of Lifestyle Disorders through Siddha system of Medicine*" on **the first Siddha Day** held on **04.01.2018** – organised by Central Council for Research in Siddha (CCRS) jointly with Directorate of Indian Medicine and Homoeopathy, Govt. of Tamil Nadu, The Tamil Nadu Dr. M.G.R. Medical University and National Institute of Siddha.

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Certificate. No: FSD/Part/ 058

# Government Siddha Medical College

**Arumbakkam, Chennai – 600 106**

## CERTIFICATE

Certified that the samples submitted for identification by Dr.R.Abinaya, PG Scholar, Department of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai-600 106, were identified as:

**Ingredients of *Kaalamega Narayana Chendhooram*:**

1. Purified *Vediuppu [Potassium nitrate ]*
2. Purified *Thurusu [ Copper sulphate ]*
3. Purified *Padikaaram [Aluminium potassium sulphate ( Alum )]*
4. Purified *Vengaram [ Sodium bicarbonate* ( Borax) *]*
5. Purified *Navacharam [Ammonium Chloride ]*
6. Purified *Pooneeru [Impure Sodium Carbonate (Fullers Earth) ]*
7. Purified *Jaathilingam* [ Red sulphate of mercury ]
8. Purified *Gandhagam [Sulphur ]*
9. Purified *Kalluppu [Sodium chloride ]*
10. Purified *Rasam [ Hydragyrum ]*
11. Purified *Aritharam [Tri sulphate of Arsenic* (Yellow Orpiment) *]*
12. Purified *Manosilai [Di suphate of Mercury* (Red Orpiment) *]*

**Date:** 25/8/17

**Place: Chennai**

25/8/17

**PG Department of Gunapadam**

# C.L.BAID METHA COLLEGE OF PHARMACY

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**Thoraipakkam, Chennai – 600 097**

## CERTIFICATE

This is to certify that the project entitled, **Toxicological and Pharmacological study on KAALA MEGA NARAYANA CHENDHOORAM & CHANDAMARUTHA CHENDHOORAM** in rats submitted in partial fulfilment for the degree of **M.D. (Siddha)** was carried out at C.L.Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2016-2017. It has been approved by the **IAEC**

**No: IAEC/XLVIII/13/CLBMCP/2016**

C.L. Baid Metha College of Pharmacy CHENNAI-97 Thoraipakkam

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**IAEC Member Secretary**